# THE

# BOTANICAL GAZETTE

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P. 214, line 27, *for* embryo formation *read* embryo sac formation
P. 215, citation **17**, *for* embryo of *read* embryo sac of
P. 381, line 6, *insert* extremely *after* producing
P. 434, add legend for fig. 1: Percentage of germination in light of different in-
    tensities.

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P. 104, heading, *for* **Proteose** *read* **Protease**
P. 105, line 4, *for* proteose *read* protease
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# THE

# BOTANICAL GAZETTE

## September 1925

### MALE CELLS IN ANGIOSPERMS

I. SPERMATOGENESIS AND FERTILIZATION IN
ASCLEPIAS CORNUTI

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(WITH PLATES I–III AND TWO FIGURES)

From the beginning of the study of fertilization in angiosperms
up to the present time, so far as the writer knows, the existence of
male cells with living cytoplasm has been a debated question.[1] Con-
tradictory data about the same species have been reported, not only
by different investigators, but sometimes by the same one. Such lack
of knowledge has already been noticed (8, 25). According to the
literature and my own investigations, therefore, the existence of male
cells with living cytoplasm in angiosperms seemed to be improbable.
Not considering this question solved, however, I continued investi-
gating spermatogenesis in different representatives of angiosperms,
paying special attention to plants indicated as having male cells.

The American plant *Asclepias Cornuti*, which has been studied
many times from the embryological point of view, proved to be the
most suitable for such investigations. As will be shown later, I found
material of *A. Cornuti* which leaves no doubt about the existence in
this plant of real male cells, formed just after the laying down of
the cell plate in the body of the generative cell, and reaching the
embryo sac in a state of perfect conservation. The principal deduc-

[1] To my great regret, not all the botanical literature of the last few years has been
available for consideration.

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tions of this investigation had already been announced at a sitting
of the Society of Naturalists at Kiew in May 1919 (9), and at a
sitting of the Ukrainian Academy of Science in May 1923, and also
partly published (10). I regret that the present work, already begun
in 1912, could not have been published sooner.

The material for the investigation has been collected partly in
the Botanical Garden of the University of Kiew, and partly in the
neighborhood of Kiew, where *A. Cornuti* is to be found here and
there almost wild, and in considerable quantities. The latter ma-
terial, being better pollinated, proved to be the more favorable
for investigation. To secure a more rapid penetration of the killing
agent, parts of the flowers were carefully prepared. After the floral
envelopes had been removed, pieces were sectioned parallel to the
direction of the course of pollen tubes, the ovaries being slightly
dissected with a razor, the pollinia removed, etc.

The material secured during several periods of growth has been
killed in different ways. The most apparently satisfactory killing
agents were formalin mixtures, tried first by NAWASCHIN (26, 27),
and afterward successfully used by other botanists of Kiew (4, 21).
For the fixation of pollinia, the following mixture was used: 15 cc.
of 1 per cent aqueous solution of chromic acid, 4 cc. of 40 per cent
solution of trade formalin, and 1 cc. of glacial acetic acid. Pollen
tubes and ovaries were killed with the same mixture, but in a weaker
concentration. For this purpose the mixture was diluted with
17.5 cc. distilled water and the quantity of formalin reduced to half.
Sometimes less concentrated solutions were used. Usually the ob-
jects were plunged in the killing mixture for 48 hours, during which
time the solution was replaced once by a fresh quantity of the same
composition.

When plunging the objects into the killing agent, measures were
taken to make them sink. The results of the fixation of pollinia not
only depend on the fixing reagents, but also on the age of the pollinia;
old and rather dried ones do not fix well. Moreover, the unsatisfac-
tory results of fixation, as it seems, are often due to other accidental
causes, as the coming to the surface of the pollinia during fixation,
their adhering to the walls of the vessel, and so on. That is why of
two pollinia taken from the same portion one appears to be properly

fixed, while the contents of the cells of the other offer a homogeneous mass quite unsuitable for investigation. Care was also taken to let the fixing reagent penetrate directly to the pollen tubes.

Following fixation the material was washed in water for twenty-four hours, dehydrated, and imbedded in paraffin in the usual manner. By means of Yung's sledge-microtome, sections were prepared for the pollinia 5 $\mu$, and for the ovaries 7.5, 12, and 13.5 $\mu$ thick.

The sections were stuck on the slides by means of albumen fixative. The pollinia often loosening from the glass, REGAUD'S method of covering the fastened and paraffin free sections with a fine collodion film was used (19). The sections of the pollinia were stained for the most part with Haidenhain's iron-alum haematoxylin, followed by a staining with 0.5 per cent aqueous solution of erythrosin.

A delicate differentiation of haematoxylin with a solution of iron-alum, and of erythrosin with alcohol, affords very good preparations for the study of the development of male cells in *A. Cornuti*. This method of staining proved to be less favorable for studying the contents of the pollen tubes, and quite unavailable for investigation of the process of fertilization. After applying different methods of staining, including Flemming's, recommended by investigators of the embryology of Asclepiadaceae (12, 13), I chose one of the mixtures of PIANEZE (28) consisting of "Malachytgrün," fuchsin S., nigrosin, saturated alcohol solution of copper acetate, and water. This method of staining, first applied to plant objects by FAWORSKY (6), seems unequaled when studying male gametes during their progress within the pollen tube and in the embryo sac, showing preparations quite transparent, on condition, of course, of a proper differentiation. The latter was produced first in alcohol, and finally a more delicate one in a mixture of one portion of alcohol with two portions of xylol. In some cases, preparations stained according to PIANEZE were differentiated once more, in the meantime being stained by a clove oil solution of orange G. This method also gave excellent results. Artificial germination of pollinia was also tried, but did not give any favorable results.

The development of male gametes in the representatives of *Asclepias* has already been studied specially by GAGER (13) and

FRYE (12), who treated them quite correctly as male cells. After the studies of KÖRNICKE (18), STRASBURGER (37), and NAWASCHIN (24), however, the existence of male cells with living cytoplasm in angiosperms was rather doubted, while on the other hand contradictory data about male gametes in *Asclepias* were given by GUIGNARD (15) and SCHÜRHOFF (31).

While GUIGNARD calls the male gametes in *Asclepias syriaca* L. (*A. Cornuti* Dec.) "generative nuclei," and draws them as such on his plates in pollen grains, as well as in pollen tubes, SCHÜRHOFF (whose paper I know only from MATOUSCHEK's report) suggests that the generative mother cell containing the two generative nuclei is maintained in *Asclepias:* that is, that here a binucleate generative cell is to be found. Moreover, many peculiar details in the process of formation of male cells in *A. Cornuti*, putting beyond all doubt the effectiveness of proper male cells in this plant, were not noticed in the papers of GAGER (13) and FRYE (12) regarding the same question. Accordingly, I consider reasonable the publication of the data of my investigation, in spite of the appearance at different times of various works treating upon the same subject.

As is known, the generative cell in *A. Cornuti* still lying near the wall of the pollen grain is hemispherical or lenticular (13, 36), depending somewhat on the shape of the pollen grain wall against which it lies (fig. 1). In the same stage the tendency of sharpening the ends of the generative cell is noticed.

As has already been reported for some other representatives of angiosperms, the generative cell in *A. Cornuti*, when withdrawn from the pollen grain wall, remains united with it by a thin band of cytoplasm (fig. 2). When detached from the wall it gradually assumes the shape of a more and more elongated spindle, with much extended tail-like ends (figs. 3, 4, 5), similar to what SMITH (35) observed in *Eichhornia crassipes*, appearing round in transverse sections including the nucleus, and oval in oblique ones. Similar cases must also have been seen by FRYE (12) in *Asclepias verticillata*, although he thinks their appearance due to the gradual change of the shape of the generative cell.

The cytoplasm of the generative cell is very dense, with minute granular structure, and, if quite normal and well fixed, does not in-

clude any vacuoles. In a pollinium with non-vacuolate generative cells, several cells happened to be found whose cytoplasm was somewhat vacuolated. Sometimes in the cytoplasm small bodies are observed. They may be stained black with iron-alum haematoxylin, and happen to occupy the place peculiar to centrosomes, but I never observed figures as reported by NAWASCHIN (24) for *Lilium Martagon*, which might have had even a distant resemblance to blepharoplasts.

In contrast with the cytoplasm of the generative cell, that of the pollen grain is much vacuolized. It usually contains deeply stained bodies of protein nature (figs. 3, 13, 18a), which have already attracted the attention of GAGER and FRYE, and have recently been described in detail by GUIGNARD. The two cytoplasms differ in their relation to stains, the generative being more erythrophyllous. If using iron haematoxylin stain, therefore, followed by an aqueous solution of erythrosin, the cytoplasm of the generative cell, stained red, would distinctly project on the somewhat blue ground of the pollen grain cytoplasm. The outer layer of the latter, however, quite distinct where in contact with the cytoplasm of the generative cell, is also stained a shade of red which is conspicuous in cases of the parting of the two cytoplasms from each other (figs. 5, 16). In the same way the upper layer of the cytoplasm of the pollen grain adjacent to the wall is often stained. I did not chance to observe a layer of the generative cell confining it from without and staining otherwise than the inner portion of its cytoplasm, this quite agreeing with the data of NAWASCHIN (24) concerning *Lilium Martagon*.

In treating the division of the generative nucleus, which takes place within the pollen grain, I find it reasonable to note that although *A. Cornuti* offers a helpful object for the study of the formation of male cells, it is quite unnecessary here to investigate the details of the nuclear division, especially because of the extremely small size of the chromosomes, which has been referred to in the literature (13, 36). For this reason I have studied only the chief features of the nuclear division of the generative cell.

During the growth of the generative cell, its nucleus gradually passes into the prophase of the division. This phase, in its early and later stages, has often been observed in my preparations, one of them

being shown in fig. 4. In this figure a somewhat curved generative
cell is to be seen, with its nucleus in late prophase.[2] The generative
nucleus, with still prominent outlines, includes a slightly stained
nucleolus and twelve already individualized chromosomes, appear-
ing in most cases as short and somewhat curved rods. It must be
noted, however, that during the prophase two-limbed and U-shaped
chromosomes were sometimes observed. After careful and extended
counting, the number of chromosomes for the haploid nucleus agreed
with GAGER and not with STRASBURGER (36).

More seldom I met with the metaphase of the division of the
generative nucleus. This circumstance, as well as the rarity of mito-
tic figures, has already been noted by GAGER.[3] One of the cases of
the metaphase that I have examined is given in fig. 5. One is im-
pressed by the fact that the spindle threads do not occupy the whole
generative cell, but only its middle part, and their focusing at the
poles is scarcely defined; the much extended ends of the genera-
tive cell consist of granular cytoplasm. Such a structure during
metaphase has often been found in my preparations.

That similar cases seem to have been observed by WYLIE (39) in
*Elodea canadensis* is inferred from his figures, but nothing is said
about it in the text. A complete contrast with the conditions I saw
is presented by GAGER's data for *Asclepias Cornuti* and FRYE's (12)
for *A. verticillata*, who figure generative cells in the metaphase stage,
with the whole of their bodies occupied with filaments focusing at the
poles and without any granular cytoplasm within. It is interesting
to note that STRASBURGER (37) emphasizes this fact in his paper,
where, referring to GAGER's figure, he says (p. 518):

Eine andere Figur zeigt, dass in der generativen Zelle, die sich schon vor der
Schlauchbildung verdoppelt, das gesamte Cytoplasma in der Spindelbildung
aufgebraucht wird. Nur ein inhaltsleerer Raum umschliesst diese Teilungsfigur.

FRYE does not give any explanation of his figure, while GAGER
states as follows (p. 138):

The nuclear spindle in this division is different from those observed in any
of the other divisions. It is rather sharply pointed at both ends, and much longer

[2] In the previous investigations of the spermatogenesis in *A. Cornuti* referred to
nothing is mentioned about the prophase of the division of the generative nucleus.

[3] This is generally said about the division of the generative nucleus; in GAGER's
paper only one figure of metaphase is given.

and more acuminate at one pole than at the other (fig. 32). The more tapering
pole appears to be somewhat bent to one side, but this was doubtless caused by
the shoving of the microtome knife, or by some other mechanical injury. The
difference in the length and manner of tapering of the two ends does not seem
to be due to the way the section was cut, as the poles seem well defined and
pointed at both ends, and not truncate at one end as would be expected had
the microtome knife cut off one pole. Here also the nucleus was too tiny to
permit of the details of spindle formation, etc., being clearly made out.

Besides this difference of GAGER's observations and mine, his
words show that he closely approached the question of the structure
of the ends of the generative cell, nevertheless attributing the little
bend of one of its poles to an artifact, while the unequal tapering
of both ends, in his opinion, does not depend on the direction of the
slide. My observations proved that the question might be solved in
a way somewhat differing from GAGER's.

As already mentioned, during the growth of the generative cell its
ends become very elongate, assuming a tail-like shape (figs, 4, 5, 12).
These elongated ends wind and often dispose themselves in differ-
ent planes. They are often cut off with the microtome knife from
the middle portion of the generative cell, and, as they represent very
fine cytoplasmic filaments, they are seen only if very well fixed
and stained. This probably explains why none of the investigators
of spermatogenesis in *Asclepias* mentions these tail-like projections
of the generative cell, giving, as will be shown later, a peculiar shape
to the male cells and sometimes rather obscuring the real structure
of the male gametes. In rather rare cases when I happened to ob-
serve the generative cells in metaphase of their nuclear division,
they usually were supported with an arclike outgrowth (fig. 5); but
on comparing the earlier and later stages of development, I conclude
that here both outgrowths must exist, the two being scarcely dis-
tinguished because of their winding.

Comparing GAGER's data with mine, it may be thought that he
figures the generative cell with its outgrowths cut off; whereas the
longer and somewhat curved end of it mentioned by him is merely
the base of one of them. In the stage of equatorial plate I counted
9–12 minute chromosomes, the splitting of which I did not succeed
in seeing (fig. 5).

According to GAGER (13), during its nuclear division the gen-

erative cell is surrounded by a wall, persisting even later around the male cells. FRYE also reports similar walls in the pollen grains of *A. verticillata*, but according to his observations they are not always conserved to the end of the division of the generative nucleus. Upon comparing the figures of both writers with what I have seen in my preparations, I came to the conclusion that the walls are the external (membranous) layer of cytoplasm of the pollen grain adjacent to the body of the generative cell. Due to its staining similar to that of the cytoplasm of the generative cell (at least in the staining I used), and on the other hand differing from the rest of the pollen grain plasma, this layer is especially obvious in places, where because of the pressure the two cytoplasms are conspicuously detached from each other. If both cytoplasms are quite close to each other, this layer is more difficult to observe, if in general the observation be possible (figs. 1, 3, 4, 5, 8, 16). These causes and the difference in staining of preparations might probably explain the fact that FRYE observed the membranes round the generative cells at one time but not at another. Sometimes, if well stained, the outer layer of the pollen grain cytoplasm becomes very prominent, and makes the impression of a frame bordering the cavity which includes the generative cell or the male gametes resulting from it. This frame, in certain cases suggesting a cell wall, might deceive the investigator. Such cases are to be seen in GAGER's figs. 32 and 33, and also in my fig. 16.

GAGER and FRYE do not mention the anaphase of the division of the generative nucleus. This stage appeared now and then in my preparations. As in metaphase, the middle part of the generative cell is occupied by spindle threads, the edges consisting of granular cytoplasm sometimes including separated vacuoles (fig. 9). In this figure it is also obvious that an outer protoplasmic layer in the central portion of the generative cell has also a granular structure. It must be noted that the fine structure of the cytoplasm of the generative cell may be seen distinctly if well fixed and stained. It happens that the generative cells of adjacent pollen grains of the same pollinium do not display equally the organization of their cytoplasm. Such a case is given in figs. 8 and 9. The first, being drawn from a casually overstained generative cell, does not give any idea of the real structure of its cytoplasm, appearing homogeneous.

During the anaphase of the division of the generative nucleus, I chanced to observe a very interesting phenomenon. In some of the generative cells I found the normal haploid number of chromosomes for *A. Cornuti*, that is, for two sperm nuclei twenty-four chromosomes separating toward the opposite ends of the cell, as is to be seen in figs. 6a and 6b, drawn from two successive oblique sections of a generative cell. In other generative cells in the same pollinium I counted half the normal number of chromosomes, that is only twelve chromosomes destined for the two sperm nuclei. Such cases are given in figs. 8 and 9, the uncommon distribution of the chromosomes about the whole cell being noticeable in the first one. Consequently two kinds of sperm nuclei may arise in a pollinium, one containing twelve chromosomes and the other six. As such mitotic figures are not of common occurrence I could not explain the cause of this event, but probably either a double reduction takes place, or during the separation the chromosomes fuse two and two. Probably the second supposition is right, their size being approximately twice the normal, which is noticeable in comparing figs. 6a and 6b with figs. 8 and 9, drawn to the same scale.

In my preparations the chromosomes of the anaphase have a peculiar appearance, often appearing two-limbed or even as if consisting of two globules with a little chink between them (figs. 6, 8, 9). Sometimes they were elongate or ∪-shaped, which is to be seen in figs. 7 and 8 (one of the upper chromosomes). Besides, it is obvious that the ∪-shaped chromosome in fig. 7 is somewhat narrowed in its middle part. As already stated, I have observed such chromosomes many times in the prophase. Sometimes they were so narrowed in their middle part as to seem to consist of two lightly oblong limbs. It is obvious that if the limbs are somewhat rounded, besides noticing a certain orientation of chromosomes in the body of the generative cell, the chromosomes can assume the shape observed in my preparations. This explanation agrees with GAGER's data suggesting the chromosomes of *A. Cornuti* as ∪-shaped, and figuring them sometimes with enlarged ends.[4] It is interesting that according to SAKAMURA's (29) fig. 9, even much longer ∪-shaped chromosomes

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[4] It will be noticed from his figures that GAGER observed such chromosomes, not in the division of the generative nucleus, but during the division of the pollen mother cells.

of *Vicia Faba* happen to assume altitudes reminding one of the appearances in the anaphase of the generative nucleus division in *A. Cornuti* already referred to.

Passing to the telophase stage, I shall emphasize the fact that this stage is accompanied by an interesting process of formation of the cell plate, which from my point of view is of great importance in reference to the question of the existence of male cells in *A. Cornuti*. During the progress into the telophase, the cell plate always becomes more and more prominent, being especially well seen between the sperm nuclei passing into the resting stage (figs. 10, 11). Fig. 11 shows a somewhat later stage of the formation of male nuclei, as may be concluded from their larger size and the prominence of their nucleoli.

The cell plate was constantly observed during the late telophase, but, as this stage seems to be of short duration, the number of possible observations was not great. As shown in figs. 10 and 11, a well pronounced, though very delicate, cell plate is laid down in the middle of a typical barrel-shaped fragmoplast, consisting of quite fine spindle threads. In the first of the mentioned figures the fragmoplast is spread across the whole generative cell, while in the second one it is narrower than the cell. Here, as in the earlier stages of the division of the generative nucleus, the fragmoplast does not occupy the whole generative cell. Male nuclei, being mostly spherical and considerably less in size than the generative nucleus they are derived from, soon after their formation enter into the resting stage. Each of them includes one nucleolus, their chromatin appearing at this time as granules of different sizes. It results from my observations, therefore, that the ability of the cytoplasm of the generative cell of *A. Cornuti* to assume a fibrillar structure during its nuclear division, occupies an average position between the abilities that GAGER (fig. 32) and FRYE (fig. 25) state for 12 representatives of the same genus, and the data of NAWASCHIN (24) for *Lilium Martagon*, where no fragmoplast is formed.

Before discussing the formation of male cells, I shall mention with more detail the question of laying down of the cell plate, which precedes the formation of male gametes, not only in *A. Cornuti*, but also in some other representatives of seed plants. No data have been

found on this subject by the investigators of spermatogenesis in *Asclepias*, although in FRYE's (12) paper there is a figure (fig. 26) similar to my figs. 10 and 11, but no traces of any cell plate are shown; and nothing is said about it in the text. FRYE's figure generally seems much schematized, and does not give any idea of the rather subtle structure of the generative cell during the laying down of the cell plate. Very scanty data concerning the question under discussion are to be found in literature. Not only in angiosperms but even in gymnosperms the laying down of the cell plate before the formation of male gametes very seldom happened to be observed, the process being of rather short duration. The previously known cases may be divided into two groups: either the formation of the cell plate is not followed by an appearance of separate male cells, or the latter appear as quite independent structures.

Among the gymnosperms, *Pinus austriaca* belongs to the first group. In this species, according to FERGUSON (7), "No cell wall is ever formed, and in only one instance was a condensation of the spindle threads in the region of the cell plate observed." This is also illustrated by NAWASCHIN and FINN (25),[5] and in *Picea excelsa*, where MIYAKE (20) observed the appearance of the cell plate which afterward disappears (see also NAWASCHIN and FINN).

Among the angiosperms, *Lilium Martagon* can be referred to a certain grade in the same group, although NAWASCHIN (24) suggests that generally no fragmoplast is formed in the cytoplasm of the generative cell; yet STRASBURGER (37) did observe it in some cases with a rather weakly developed cell plate. To the same group must also be referred *Neottia Nidus-avis*, where, according to MODILEWSKI (21), in the plasma included between the sperm nuclei, a laying down of a fragmoplast with a delicate filmlike evanescent cell plate is observed; and probably *Ruppia* also, where the division of the generative nucleus is followed by a laying down of a rather fine cell plate, as was shown by MURBECK (22) and GRAVES (14).

There are still fewer representatives of seed plants for which data enough exist to place them in the second group. *Taxus canadensis*, studied by DUPLER (5), may of course be referred to this category.

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[5] It is of interest to note that the fragmoplast with the cell plate in the figure of FERGUSON is similar to that observed in *A. Cornuti*.

DUPLER states that during the division of the body cell, "A broad spindle is formed, and the cell plate laid down on it is lenticular in outline, resulting in the formation of the two unequal male cells, a small lens-shaped cell, and a larger more rounded one."

As to the angiosperms, no definite data concerning this question have been given for any representatives, which has partly been noted by NAWASCHIN and the writer (25), and, so far as I know, we do not find in recent work any information about the laying down of the cell plate before male cell formation in angiosperms (see WYLIE 40, SCHÜRHOFF 31, 32, HERRIG 16, 17, SAWYER 30, TISCHLER 38, and SHARP 33).

*A. Cornuti*, therefore, appears to be the only angiosperm which can be assigned to the second group, for doubtless cell plates are repeatedly found before the formation of male cells. This process is perhaps of much more common occurrence among seed plants, but it has escaped investigators for various reasons.

After the cell plate is laid down, the separation of male gametes begins, a light appearing in the middle of the generative cell, revealing a slight chink between the newly formed male cells (figs. 17*a* and *b*, 12, 13). The division of the protoplast of the generative cell begins before the last traces of spindle fibers have disappeared; it takes place in the line of laying down of the cell plate, a denser wall, as it seems, not being formed. This will be discussed later. Usually I happened to observe cases where the separation of male cells had already occurred, but sometimes I succeeded in observing the actual process of division of the protoplast of the generative cell into two male gametes. A similar case is represented in fig. 17*a* and *b*, drawn from two subsequent sections of a generative cell. While in the first (*a*), where we have the uppermost portion of the cell, the parting protoplasts are still joined together by a fine cytoplasmic strip, in the second (*b*) they are quite independent.

Generally the surfaces of the separating male cells facing one another are not identical, one of them being often somewhat convex, while the other is concave (figs. 12, 13, 17*b*). Sometimes, however, these surfaces are corrugated (fig. 14*a*), and, if badly fixed, they become covered with jaggs and cavities, which give an impression that one male cell has drawn out one or many protoplasmic jaggs of the sister cell.

Male cells are almost mature when in the pollen grain, and with their tail-like projections they suggest bullheads or sperms of some organisms, as seen in figs. 12, 13, 14*a*, 17*b*, 18*a*, and 19. Comparing these figures, it is obvious that the shape of sperms varies within certain limits, perhaps partly depending upon their age. The broadened portion of the male cell, corresponding to the head of a spermatozoid, is either nearly spherical, or heart-shaped, pear-shaped, hornlike, etc.; in several cases it becomes narrow-tailed at once (figs. 12, 19), while in others it gradually assumes this shape (figs. 18*a* and *b*). In considerably elongated male cells sperm nuclei also assume an oblong form (figs. 13, 18*a* and *b*). The tail-like projections of sperms, showing, as already noted, much extended ends of the generative mother cell, vary somewhat, especially differing in their length and size (figs. 12, 18*a*, 19).

Sometimes male cells assume a very queer shape, as for instance the left one in fig. 18*a*, resembling a hammer-headed shark, or the right one in the same figure, twisted under the nucleus. In some cases the separation of male cells takes place when the generative cell is still slightly curved (figs. 12, 13), while in others (fig. 17*a* and *b*) it occurs when the generative cell is curved in a bow. Sperms when formed often settle, however, forming different angles (fig. 14*a*); sometimes they curve to such a degree that the middle portions of their bodies become almost parallel, and the ends, lying one upon the other, form a kind of closed arc. Such a case is shown in fig. 18*a* and *b*, where one adjusting of the microscope shows the sharpened ends of male cells (*a*), while the other (*b*) gives the impression of an almost closed arc. A more interesting case is fig. 16, where the tail-like projections lie so strictly one upon the other that by different adjustings of the microscope their ends cannot be distinguished, while only one definite adjusting shows a scarcely visible chink, showing this arc to be a result of the two tail-like projections lying one upon the other. When at the beginning of my investigation I saw this preparation, I thought that a binucleate generative cell, very narrowed in its middle portion, would be formed, but further study showed that this conclusion was wrong.

The variety of the observed cases increases because the enlarged parts of male cells, as if corresponding to the head of a spermatozoid, are sometimes supplied with beaklike outgrowths, and, if lying one

upon the other, an arched vault appears between the gametes (fig.
14*a* and *b*). The same male cells are represented by different adjust-
ings of the microscope; by the first the male cells are quite inde-
pendent (*a*), while by the second (a beaklike projection appearing
by the upper cell and reaching the lower one) they seem to be re-
united with a streak of male cytoplasm (*b*). If simultaneously much
extended ends of male cells overlie one another, this combination
results in a kind of ring. Nevertheless, it could be noticed in all the
preparations that male cells become individualized after their forma-
tion while yet within the pollen grain. I have not met with the solid
connection between the male cells observed by WYLIE (39, 40) in
*Elodea canadensis* and *Vallisneria spiralis*, and by SHATTUCK (34) in
*Ulmus americana* (see NAWASCHIN and FINN 25). These cases of
change in shape and disposition of male cells do not exhaust all the
diversity of appearances which are to be seen, but they give a suffi-
cient idea of it.

The cytoplasm of male cells preserved in my preparations the
structure peculiar to the cytoplasm of the generative cell, but occa-
sionally somewhat enlarged male cells appeared, with a vacuolated
cytoplasm. These sperms have an extraordinary aspect, their cyto-
plasm reminding one of the cytoplasm of pollen grains (fig. 15).

GAGER'S (13) and FRYE'S (11, 12) papers include no data which
would give a clear idea of male cells in *Asclepias*. The best figured
by GAGER is in fig. 33, representing, as it seems, male cells with
badly secured cytoplasm in its transverse section, almost like my
fig. 20. On the contrary, male cells of *Vallisneria spiralis*, figured by
WYLIE (40), are very like the corresponding elements of *A. Cornuti*,
but deprived of the long outstretched ends so typical for the sperms
of the latter.

Occasionally some original figures were found, one of them being
given in fig. 16. Here male cells, under the influence of the fixing
reagent, have shrunk and parted from the cytoplasm of the pollen
grain. Between the male cells a partition is seen, which stains with
erythrosin rose. This partition, by its ungranular structure and
mode of staining, is very like the thinnest limiting layer of the pollen
grain cytoplasm, closely adjacent to the cavities, including the male
cells. Figures similar to the latter are somewhat like those that

CHAMBERLAIN (**1, 2, 3**) has observed during the spermatogenesis in *Dioon edule*, *Ceratozamia mexicana*, and perhaps in *Stangeria paradoxa*. According to him, the spermatozoids of these cycads are formed within the two cells (mother cells) resulting from the division of the body cell. After the breaking down of the partition between the two spermatozoids and the wall of the body cell, the spermatozoids are set free.

As to the partition sometimes observed between the male cells in *A. Cornuti*, I have no direct evidence of its genetic relation with the cell plate appearing, as we have seen, in the telophase of the division of the generative nucleus. More credible appears the suggestion, based on the study of numerous preparations, that the partition represents a very fine membrane of the plasm of the pollen grain, which has penetrated between the male cells after their separation. At a considerable shrinking of male cells the membrane becomes especially prominent, as seen in fig. 16. Whatever may be the shape and disposition of male cells, they remain close together while within the pollen grain, so that their conjunction conserves the outlines of the mother generative cell, as shown in figs. 4, 12, 13, and others.

Much more difficult is it to observe well conserved male cells within the pollen tubes, but I succeeded in obtaining a series of preparations, mostly stained according to PIANEZE, which made possible the study of the details of the structure of the male cells during their progress within the pollen tube. Here they remain either close together, a fact already noticed by GAGER, or they follow after one another at a more or less considerable distance, as shown in figs. 21, 22, 23, and text figs. 1 and 2. In the first case sometimes there is no layer of pollen tube cytoplasm between the male cells (fig. 21 and text fig. 1). Sometimes sperms, during their progress through the pollen tube, are separated from one another at a considerable distance, as shown in fig. 23, where the second gamete, which is not figured, has become much separated.

In *A. Cornuti* I did not meet either the considerable variations in size, or any connection between the male cells, which WYLIE (**40**) observed in *Vallisneria spiralis*. In *A. Cornuti* the growth of the sperms during their progress through the pollen tube is rather insig-

nificant. Sometimes I happened to observe the presence of the tail-like projections of the male cells (fig. 23), in such cases the latter being very like swimming male gametes of some organisms. The tail-like end is either turned backward (fig. 23), or it may be in front. In the cases where the male cells are close together, their much extended ends are directed to opposite sides, as in pollen grains.
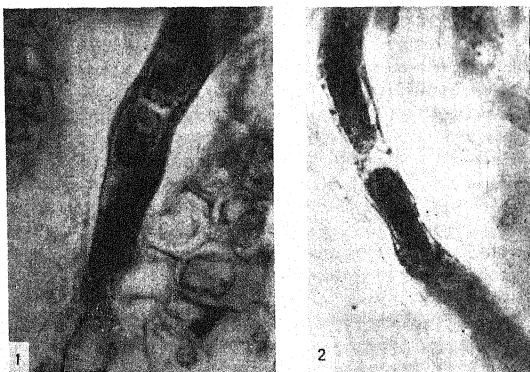


FIGS. 1, 2.—Photomicrographs: fig. 1, portion of pollen tube proceeding through ovarian chamber (pollen tube is in corner, formed with placenta and funiculus); male cells quite close together; portion of upper one not in section; on lower right, placenta tissue; on upper left, tissue of integument; fig. 2, portion of pollen tube within upper part of style; male cells at little distance from one another, their protoplasts also obviously limited from pollen tube cytoplasm.

My preparations do not suggest the least doubt that the bodies destined for fertilization in *A. Cornuti*, during their progress through the pollen tube, are real male cells. The cytoplasm of the latter, conserving in the pollen tube its small granular structure and its peculiar relation to stainings, differs from the cytoplasm of the male cells when within the pollen grain only in somewhat more accentuated vacuolization. In successful preparations, stained according to PIANEZE, the cytoplasm of male cells, somewhat loosened from the

plasma of the pollen tube, is conspicuous by its light red staining of
the dark pollen tube contents (fig. 21 and text fig. 1). When stain-
ing with iron-alum haematoxylin, followed by the staining with an
aqueous solution of erythrosin, one sometimes succeeds in staining
the male cells a deeper red than the cytoplasm of the pollen tube
(from such a preparation are made fig. 22 and text fig. 2). The latter
appearing coarse grained, conserves to a certain degree its friable
reticulate structure, which makes it differ strongly from the small
grained cytoplasm of male cells (fig. 22).

It is interesting that, in GAGER's (13) opinion, traces of the deli-
cate membrane of the generative cell remain for some time around
the male cells while within the pollen tube. Evidently he is speaking
of the finest limiting layer of the cytoplasm of the pollen tube closely
adjacent to the male cells, as previously discussed. During the prog-
ress of male cells within the pollen tube, their nuclei remain in the
resting stage, perserving the spherical shape, the nucleoli, and the
chromatin in granules (figs. 21, 22, 23, and text figs. 1, 2). The tube
nucleus, spherical within the pollen grain, becomes somewhat elon-
gated while within the pollen tube, either preceding the male cells,
or following them (fig. 22).

For a long time I could not succeed in observing the male cells
in the moment of emergence of the pollen tube contents into the
embryo sac, or even the next moment after it. One of the causes
of this failure is the short duration of the events of fertilization, with
the exception of the very fusion of the copulating nuclei, the latter
having drawn already the attention of NAWASCHIN (23) and later
of WYLIE (40).[6] Moreover, the considerable quantity of discharged
material from the pollen tube, having a special affinity for stains,
prevented the obtaining of good, well differentiated preparations,
in which one might distinguish the male gametes from the remain-
ing contents of the pollen tube and embryo sac. Occasionally I ob-
served a male gamete within the pollen tube directly after the pene-
tration of the latter within the embryo sac (fig. 24). Before fixation
one of the ovules had been intersected so as to leave only the micro-
pyle pole of its embryo sac; this made all its contents fall out, where-

[6] In order to retard the events of fertilization, WYLIE washed the ovules of *Val-
lisneria spiralis* in ice water and fixed them with a very cold killing agent, this method
giving excellent results.

as the tip of the pollen tube, already within the embryo sac, remained undisturbed. As seen in fig. 24, the tube has already burst, and has evidently discharged a considerable portion of its contents into the embryo sac. It became possible, therefore, to observe the sperm stuck within the tube, having the aspect, not of a naked nucleus, but of a complete cell.

After a long series of failures, I succeeded once in observing male gametes soon after their penetration into the embryo sac, by using PIANEZE's staining followed by the staining with clove-oil orange solution, differentiating with alcohol and its mixture with xylol. The cytoplasm of male cells stained red was definitely seen on the light green ground of the emerged contents of the pollen tube. In fig. 25 the sperms are seen lying quite near to one another, as is the case in the pollen grains or sometimes in pollen tubes. Owing to the fact that here the male cells still lie one upon the other, their union might be mistaken for a binucleate cell, if the history of the development of male gametes in *A. Cornuti* were not known. The male cytoplasm preserves its peculiar structure here also. It is to be noticed that, if stained according to PIANEZE, its capacity to stain red does not change, while the aptitude of the cytoplasm of the pollen tube for staining green increases after entering the embryo sac.

It is a matter of interest that, while the male cytoplasm of *A. Cornuti* stains very well, in some species of *Juglans*, studied by NAWASCHIN and the writer (25), it remained colorless; the latter being already noticed by WYLIE (40). In vain does he suggest, however, that in *Juglans* the presence of sperm cytoplasm, surrounding male nuclei within the embryo sac, has not been exactly proved. He is led to this conclusion by the fact that in our drawings the structural presence of sperm cytoplasm is not represented; in place of it a clear space is left. On account of it I feel obliged to explain that in our paper in several places it is stated that the sperm cytoplasm in the studied species of *Juglans* is homogeneous, and, by modes of killing and staining used, remains colorless, so that it is represented in the drawings as a clear space.

I did not observe the penetration of sperm cytoplasm into the egg cell, as did not the other investigators of the sexual process in angiosperms, WYLIE included (40), so that this question and its re-

lated problems remain unsettled. The male nuclei in *A. Cornuti* preserve their spherical shape, their nucleoli, and chromatin in granules in the embryo sac. They have a similar structure immediately after the penetration of male cells within the embryo sac, and also later, directly before the fusion with the female nuclei (figs. 25–28). GAGER (13) also figures the male nuclei of *A. Cornuti* in pollen grains and pollen tubes similar to those observed in my preparations (figs. 33, 35, 36, 37), but his paper gives no information about the structure of sperms while within the embryo sac. It is quite incomprehensible, therefore, what FRYE (12) saw in the embryo sac of this plant. In fig. 48 of his paper an embryo sac of *A. Cornuti* is seen in the very moment of double fertilization. One sperm flattens against the nucleus of the egg, while the other is close to the antipodal polar nucleus. Although these sperms are bereft of all traces of their own cytoplasm, FRYE calles them "male cells," and states that "the male cells are crescentic in form, reticulated with granules, and not conspicuously unlike in size." Striking is the great difference in structure and shape of sperm nuclei figured by FRYE, and those which I observed in the embryo sac of the same plant. The "male cells" of FRYE, according to his drawing, are very like the vermiform sperm nuclei of some Liliaceae (see, for example, NAWASCHIN 23), only being of considerably smaller size. As these, they are deprived of nucleoli, their chromatin being reticulate.

According to my observations, the second sperm nucleus generally fuses with the fusion nucleus (figs. 26–30), whereas, according to the data of FRYE, it unites in most cases with the antipodal polar nucleus.

The existence of true male cells, therefore, before the formation of which a cell plate is laid down, seems to me quite proved in *A. Cornuti*. The existence of male cells in this species, and also in *Vallisneria spiralis*, makes their occurrence among other angiosperms quite probable; this should be determined by further investigation. In connection with the new data, secured during the last decade, the scheme appended to the publication of NAWASCHIN (25) and of the writer, figuring the male gametes of seed plants, must be somewhat changed and completed by including male cells among the sperms occurring in angiosperms.

## Summary

1. From the beginning of the study of fertilization in angiosperms, the question of the existence of male cells with living cytoplasm has remained in doubt.

2. True male cells exist in *Asclepias Cornuti.*

3. During the telophase of the division of the generative nucleus, a very delicate cell plate is laid down in the middle portion of the fragmoplast. The latter occupies only the middle portion of the generative cell, whereas its much extended ends consist of granular cytoplasm.

4. The separation of male cells occurs where the cell plate is laid down.

5. Male gametes reach almost maturity while within the pollen grain, and being supplied with tail-like projections suggest in their general aspect bullheads or spermatozoids of some organisms.

6. The male cytoplasm differs considerably in its structure and relation to stains from the cytoplasm of the pollen grain, being therefore well delimited from the latter.

7. Following their formation, male cells remain in the pollen grain, although quite close to one another, but yet not united, contrary to what has been described for some representatives of angiosperms.

8. The tail-like projections of male cells arise from much outstretched ends of the generative cell.

9. Within the pollen tube male cells sometimes remain close to one another, and sometimes separate a more or less considerable distance, conserving their peculiar structure and relation to stains.

10. The tail-like projections of male cells are conserved while within the pollen tube.

11. Male gametes just after their penetration into the embryo sac maintain in their structure the unchanged cytoplasm. According to PIANEZE, the latter stains red, while the cytoplasm of the pollen tube emerged into the embryo sac stains green.

12. Like the other investigators of the sexual process in angiosperms, the writer did not succeed in observing the penetration of male cytoplasm in the egg.

13. The sperm nuclei, from the moment of their formation up to

their fusion with the female nuclei, conserve their spherical shape and resting stage. The writer has never observed them to be vermi-form, as shown in FRYE'S fig. 48 (12).

14. The existence of male cells on the one hand in *Asclepias* and on the other in *Vallisneria*, as has recently been shown by WYLIE (40), makes very probable their occurrence among other angiosperms.

15. In connection with the new data secured during the last decade, the scheme appended to the paper of NAWASCHIN and of the writer (25), figuring male gametes of seed plants, must be somewhat changed and completed by including male cells among the sperms in angiosperms.

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## EXPLANATION OF PLATES I–III

All figures were drawn on the level with the microscope table, with the aid of an Abbé camera lucida. Nos. 22, 24, 25, and 26 with a Zeiss apochromatic imm. objective 3 mm., ap. 1, 3, and compens. ocular 6; nos. 4 and 18 with a Koristka apochromatic imm. objective 1.5 mm. and compens. ocular 6; nos. 6, 7, 8, and 9 with a semiapoch. imm. objective of Koristka $\frac{1}{16}$ and compens. ocular 8; all other figures with the semiapoch. imm. objective of Koristka $\frac{1}{16}$ and compens. ocular 8.

*PLATE I*

FIG. 1.—Portion of pollen grain, showing generative cell still affixed to pollen wall and tube nucleus; protein bodies within pollen grain cytoplasm.

FIG. 2.—Portion of pollen grain, showing generative cell parting from pollen grain wall.

FIG. 3.—Portion of pollen grain contents; generative cell soon after parting from wall; cytoplasm of pollen grain, showing spindle-shaped protein bodies.

FIG. 4.—Portion of pollen grain containing generative cell with nucleus in late prophase; twelve chromosomes seen; tube nucleus on left.

FIG. 5.—Preparation similar to fig. 4, but nucleus of generative cell in metaphase; upper tail-like end of generative cell lying upon tube nucleus.

FIG. 6*a, b*.—Two successive, somewhat oblique, transverse sections of same generative cell with nucleus in anaphase stage: *a* showing nine and *b* showing fifteen chromosomes.

FIG. 7.—Oblique section of generative cell with its nucleus in anaphase.

FIG. 8.—Portion of pollen grain, showing generative cell in longitudinal sec-

tion and tube nucleus; anaphase of nucleus of generative cell, showing peculiar disposition of chromosomes through whole cell.

FIG. 9.—Portion of pollen grain, showing longitudinally dissected generative cell with nucleus in anaphase; as in fig. 8, number of chromosomes is half the normal, their size being approximately double that shown in figs. 6*a* and *b*, where number of chromosomes is normal.

FIG. 10.—Portion of pollen grain, showing nucleus of generative cell in late telophase, and tube nucleus; fragmoplast with obvious cell plate is seen; ends of generative cell, consisting of small granular cytoplasm, are partly cut off.

FIG. 11.—Stage of division of generative nucleus, similar to that shown in fig. 10, but fragmoplast does not occupy whole diameter of generative cell.

*PLATE II*

FIG. 12.—Male cells immediately after their formation, surrounded with pollen grain cytoplasm.

FIG. 13.—Preparation similar to fig. 12, but male cells more elongated, tail-like outgrowths not visible; sperm nuclei also oblong; close by tube nucleus a group of protein bodies; the figure being made from a well fixed preparation, neither are male cells loosened from pollen grain cytoplasm, nor is latter loosened from pollen grain wall.

FIG. 14*a*, *b*.—Same male cells by different adjustings of microscope: *a*, wholly separated male cells with streak of cytoplasm of pollen grain between their enlarged ends; *b*, foremost portions of same male cells; owing to appearance of beaklike outgrowth at upper sperm cell, male gametes apparently united by strip of male cytoplasm.

FIG. 15.—Male cells with vacuolated cytoplasm, showing extraordinary appearance; bit of pollen grains cytoplasm squeezed between male cells.

FIG. 16.—Portion of pollen grains, showing male cells with protoplasts shrunk and parted from pollen grain cytoplasm; ends of male cells much extended, lying one upon the other, forming a kind of arc somewhat enlarged in middle; between enlarged parts of male cells and their outstretched ends, lying one upon the other, a bit of pollen grain cytoplasm is squeezed; on left a group of protein bodies.

FIG. 17*a*, *b*.—Two successive longitudinal sections of generative cell in moment of separation of male gametes: *a*, individualizing protoplasts of male cells still united with strip of generative cell cytoplasm; *b*, separated male cells; in both a bit of pollen grain cytoplasm passing upmost in a fine strip is squeezed between the male cells.

FIG. 18*a*, *b*.—Same male cells by different microscope adjustings, showing their fantastic shape, right cell being bent under nucleus; enlarged fishtail-like ends are close together; opposite ends of male cells, formed from much extended ends of generative cell, overlie one another: *a*, whole pollen grain and its connection with vicinal pollen grains (outstretched ends obvious); *b*, same overlying one another, giving impression of continuous arc.

PLATE I



1

2

3

4

a

6

b

5

7

8

PLATE II



12

13

15

*b*

16 *a*

*b*

*a*

14

*a*

*b*

17

18

*a*

*b*

19

20
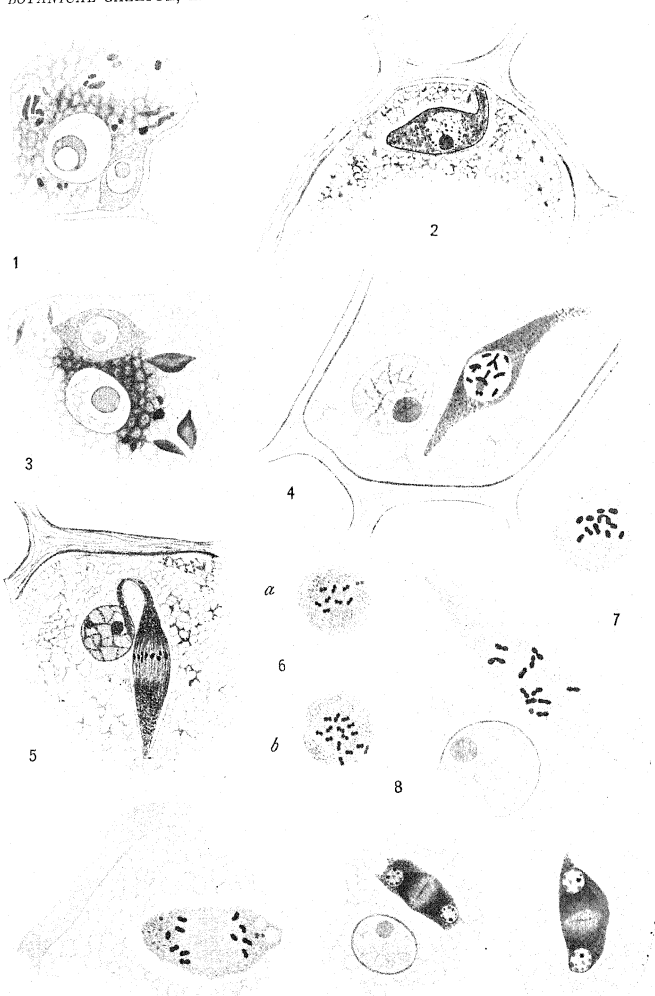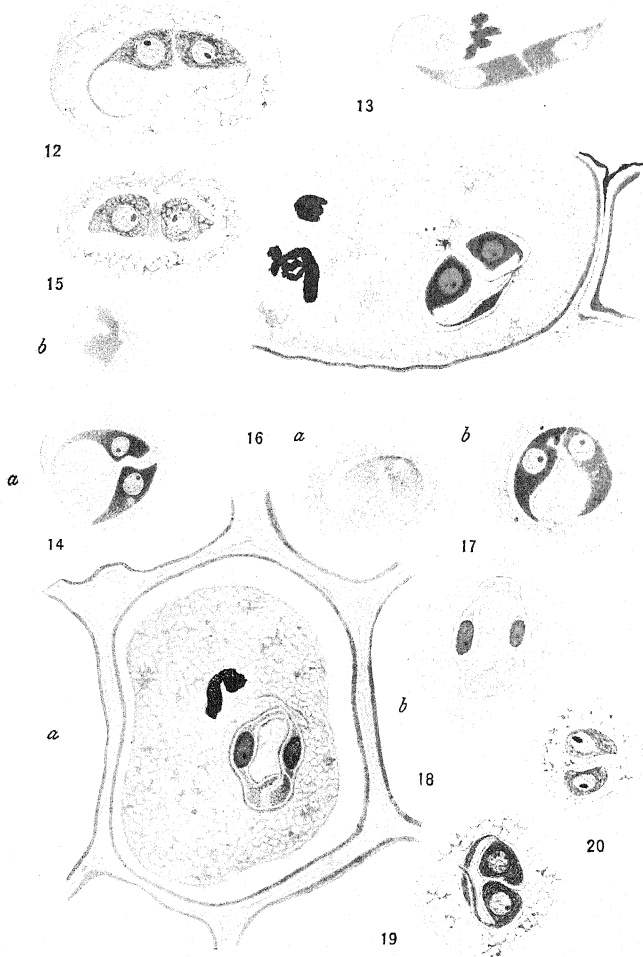
PLATE III



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23
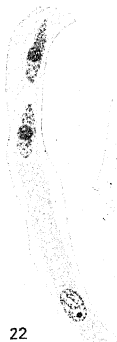
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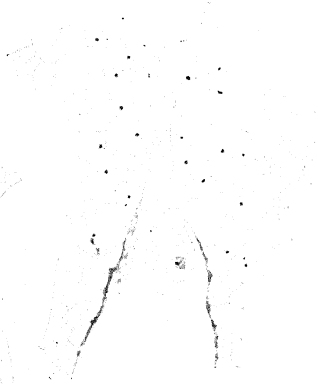
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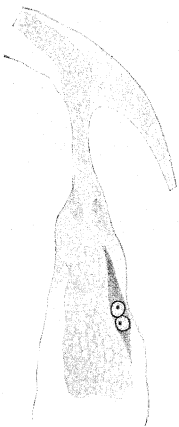
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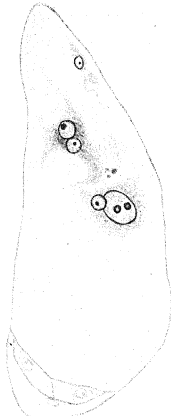
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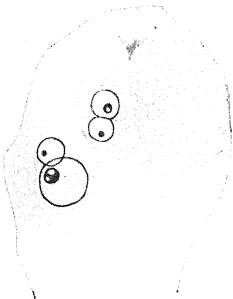
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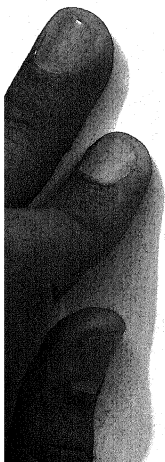
FIG. 19.—Male cells with tail-like outgrowths lying one upon the other with pollen grain cytoplasm around them.

FIG. 20.— Male cells in cross-section, with cytoplasm of pollen grain around and between them.

*PLATE III*

FIG. 21.—Portion of pollen tube passing within ovary on level with fastening of fourth ovule, counting from above; adjacent male cells separated merely by small space from one another and from pollen tube cytoplasm (lower one nearly all in section, portion of upper being cut off).

FIG. 22.—Portion of pollen tube within upper part of style; in front tube nucleus; male cells more separated than in fig. 21.

FIG. 23.—Male cell with tail-like end directed backward within portion of pollen tube passing in upper part of ovary.

FIG. 24.—Micropylar pole of ovule; contents of embryo sac fallen out except pollen tube penetrated within; latter is perhaps already burst out; male cell is stuck at its end.

FIG. 25.—Micropylar pole of ovule with pollen tube contents just emerged into embryo sac; on right male cells are close.

FIG. 26.—Embryo sac in act of fertilization; one sperm nucleus penetrated within egg and in close connection with its nucleus, second just approaching fusion nucleus; on upper right of egg the nucleus of one of synergids, lower antipodals.

FIG. 27.—Oblique section of upper portion of embryo sac, same stage of fertilization as in fig. 26; uppermost, on right of egg, emerged contents of pollen tube.

FIG. 28.—Fusion of sperm nucleus with fusion nucleus.

FIGS. 29, 30.—First nuclei of endosperm formed by triple fusion.

# THE GAMETOPHYTE OF LYCOPODIUM CERNUUM
## IN HAWAII[1]

### OTTO DEGENER

#### (WITH PLATES IV–VII AND TWO FIGURES)

Hawaii National Park, but a few hours' ride from the city of Hilo, is situated at an elevation of 1200 m. on Mauna Loa, an active volcano. Here, within an area of 10–20 sq. km., may be found every type of habitat, ranging from that of the bare glassy flows of "pahoehoe" lava in the crater of Kilauea, or the Kau Desert, to that of the humid tree-fern forest. Near the brink of the crater itself is a series of earthquake crevices from which steam continuously rises. This extends about 1.5 km. from a region of volcanic ash, upon which a few stunted plants of a peculiar Hawaiian composite and of a heath manage to exist, to an area thickly covered by an almost impenetrable tangle of vegetation. The conspicuous species characteristic of the latter luxuriant type of vegetation are the tree *Metrosideros polymorpha* Gaud.; a small tree-fern of the endemic genus *Sadleria;* *Gleichenia dichotoma* Hook., which clambers over any support to a height of 30–45 dm.; and *Lycopodium cernuum* L., which forces itself up through the underbrush and frequently rises to a height of 15 dm.

In December 1922, the writer, searching for the gametophytes of club mosses, noticed several sickly plants of *Lycopodium cernuum* at the brink of a stream crack in the area known as the Sulphur Bank. Upon observing these plants more closely, a few small sporelings were discovered on the sloping sides of the crevice. With this clue as to the type of locality in which the gametophyte of the species might be found, similar situations were investigated. Thousands of young sporelings and gametophytes were discovered, but since the circumstances under which these plants were growing can be duplicated only in a region of volcanic activity, stations not influenced by subterranean heat will first be described.

### Stations under normal conditions

Six isolated groups of sporelings and gametophytes were found growing under ordinary conditions within 1.5 km. of the Volcano House. Here the vegetation is similar to the luxuriant type just mentioned. The mean yearly rainfall is approximately 104 inches. A brief description of these individual stations will be pertinent to a question to be discussed later.

1. At the road that leads around the rim of the crater, workmen had dug out some weathered lava, leaving a hollow in the roadside several decimeters in diameter. Here seven sporelings, most of them only a few millimeters in size, were growing among fine moss.

2. On either side of a sunken woodroad leading to a tree-fern forest, more than a score of specimens were found. They were growing near the base of a sloping embankment of clay soil 5 dm. high, made by the roadcut. Most of them were in company with young mosses. An indication of the relative rapidity of growth of the gametophyte of this species, in contrast with that of *L. clavatum* and *L. obscurum*, is shown by several plants which were growing at a spot where it was very plain that the wheels of carts had sheared off the soil in passing. This station seemed drier than the station previously mentioned, and was exposed to full sunlight. Most of the plants a centimeter or more in height were dying off at the base. The younger plants appeared stunted and not thriving.

The four remaining stations were located along a railroad bed abandoned years ago by loggers. The rails had been removed, but the ties remained and had partly decayed. Their surfaces were generally level with the soil and covered by a sparse vegetation of moss and grass.

3. One of these stations was a very damp slope below an overhanging rock, and was exposed to the early morning sunlight. The few specimens found, which were commonly in the protophyll stage, were loosely imbedded in moss.

4. Another station was also on a slope, but along a shady stretch of the railway. Within an area about a meter in diameter, more than a dozen sporelings were found, ranging from a few millimeters in length to branching and rooting specimens several centimeters long. They were rooted on a bed of fine moss that had overgrown the gravelly embankment.

5. At another station a few sporelings 7 cm. or more in length were noted at the side of the roadbed in dry soil. These plants were dying, presumably from lack of moisture.

6. The last station discovered yielded by far the best material. It covered the entire width of the roadbed for a length of 10–12 m. The place was rather well shaded by the giant fronds of several individuals of a *Cibotium*, while shrubbery and thickets of *Gleichenia* were encroaching upon the roadbed from either side. The ground was unusually moist, since here the railway passed over a depression. The soil was very gravelly, due to volcanic ash, but nevertheless tenaceous, because of an admixture of tawny clay especially rich in aluminum and iron. It was generally covered by a fine mat of young moss of coarse texture, while a sedge (*Uncinia Lindleyana* Kunth), a grass, and a few small ferns were the predominating vascular plants. In the center of the roadbed, where but few of the larger plants had gained a foothold, about seventy-five gametophytes with sporelings possessing from three to a dozen leaves were gathered. Away from the center small sporelings were conspicuously absent, whereas larger ones that had branched profusely and had rooted at the nodes were the most outstanding plants.

In May 1923, a station was discovered near Honolulu, on the island of Oahu, on a wind swept ridge at an altitude where precipitation is heavy. About fifty gametophytes and sporelings were collected on a sloping moss covered embankment less than a meter in diameter. Four sporelings several centimeters in length were growing near the edge of the station, with small grasses; all the other specimens were much smaller and grew where no larger vascular plants had developed.

The method of growth of these sporelings is not that which is typical of the adult sporophyte. The first branching is initiated before a height of 2 cm. is reached (pl. VII). The resulting limbs of the dichotomy diverge almost at straight angles to one another. One branch, however, is usually slightly more vigorous than its fellow. After this first branching, a modified dichotomy simulating monopodial growth becomes apparent. Here the branches are spaced 1–1.5 cm. apart. This type of branching usually continues until the main axis attains a length of about 6 cm. The terminus then tends to push into the soil, to swell at the tip, and to take root. Even

before a root has developed, however, a shoot arises from the whitish swollen part. It commonly makes an acute angle with the main stem and continues growth in the same direction. In this manner the plant creeps for a considerable distance, the space between successive rootings becoming longer and longer.

At each of these nodes, adventitious roots, along with several shoots, soon develop, and the latter tend to sprawl over the ground in a manner similar to that of the main rhizome. One or two of these shoots commonly become detached from the main axis. This tendency to break into isolated plantlets is more noticeable at the place of origin of the sporeling. It is usual for the part directly developed from the gametophyte to die, while a few of the adjoining segments commonly die also. The remainder have a very sickly appearance, the part between the rooting "nodes" having a tendency to die first. Because this weakened condition of the sporophyte increases the nearer its point of origin is approached, it is usually difficult to trace a sexually produced plant to its source, even though the sporeling be but a few decimeters in length.

The advanced sporophyte does not exhibit such short segments, nor does it appear weak like the developing sporeling. Instead, it creeps for a considerable distance over the ground before rooting. Then a shoot, developing at the point of anchorage, rises almost vertically for 10–25 cm. before branching. One branch then continues the plagiotropic growth so characteristic of the plant, and finally roots again. The other branch of the dichotomy grows erect to a height of a meter or more in favorable situations, such as are common near the volcano, and gives off a series of smaller branches that dichotomize more or less regularly. Upon the ends of the higher ones the strobili are formed. Their habit of hanging no doubt suggested the specific name. In vigorous fruiting individuals the branching is so regular that in counting the ultimate number of branches developing at different heights on the upright stem a perfect geometrical progression is discovered.

### Stations under volcanic conditions

Most of the steam fissures are less than 15 dm. across, although some may even reach a width of 45 dm. They are commonly less

than 3 m. long because their sides have partly caved in, but some
are at least 35 m. in length. Fig. 1 gives an approximate idea of the
location of the crevices, the smaller ones being omitted. Pl. IV,
however, shows a large part of the area in which the crevices are
common. These extend from the hotel and the Volcano Observatory,
shown in the distance, to the foreground and beyond. If all the
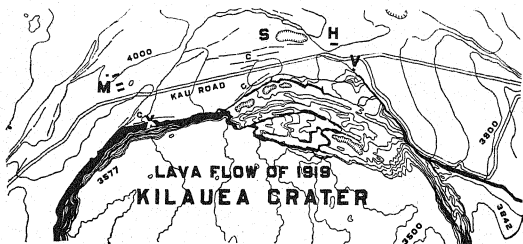


FIG. 1.—Map of volcanic region adapted from U.S. Geol. Survey, showing
steam crevices in which gametophytes of *L. cernuum* are common: *C*, volcanically
heated crevices; *H*, Volcano House; *M*, Kilauea Military Camp; *S*, Sulphur Bank;
*V*, Volcano Observatory; *X*, volcanic station depicted in pls. II and III; Scale, 1
Km.=30 mm.

steam fissures of that general region were placed end to end, their
length would total more than 2 km.[2]

The perpendicular sides of these crevices are formed almost
solely of consolidated clinkers. For many decimeters from the top
(the distance depending upon the character of the fissure and the
amount of escaping vapor) the sides are clothed by different algae
and mosses. These, intermixed at the base with a reddish clay, form
a covering sometimes as much as 3 cm. in depth. It is upon this
substratum that gametophytes and sporelings of *L. cernuum* grow
in enormous numbers.

In practically all crevices inspected, certain striking facts were
noted. Where the heat is greatest, as in the deeper part of the fissure,
plants are entirely absent. Farther toward the surface, where the

[2] Many of these steaming crevices are already obliterated with rubbish, for lack
of a more convenient place to put it.

temperature is about 40° C., a very dark sooty layer of different blue-green algae is common, the predominating one being *Fischerella thermalis* (Schabe) Gomont. This area then grades off quite definitely into one in which very dwarf mosses predominate, none of which was found in the fruiting stage. They are commonly overgrown by a thick gelatinous mass of *Gloeocapsa*. Finally, at the brink of the crevice, vascular plants become noticeable, comprising chiefly an association of *Gleichenia dichotoma* and *Lycopodium cernuum*. These plants (of which all but the largest specimen shown on pl. VII are from the crevices) always increase uniformly in height the farther they are located from the heated crevice. On the perpendicular side of the crevice itself, the lycopod specimens were imbedded in countless numbers in the layer of mosses and slimy algae. Because of the light green color of the sporelings they stood out very conspicuously, studding the moss bank every few centimeters. Not one of these sporelings on the vertical wall of a steam crevice was found to be over a few millimeters in height. Most of them had developed scarcely anything except protophylls (fig. 2), or were buried for the greater part of their height in the substratum. The temperature at their base attained a maximum of 35°, but where they grew in greatest numbers and apparently under the most favorable conditions, the thermometer stood at about 31° C. At 26° the gametophytes were rare and sickly, but sporelings a centimeter or more in length were very common. The latter were always weak and much branched, often dying in places, or even entirely dead.

Thus there was a band two or more decimeters in width, studded with gametophytes with their minute sporelings, on the smooth vertical wall of a uniformly heated crevice. Where the temperature was around 31° the plants were spaced but a few centimeters apart in many cases; at a little higher temperature they would become less numerous; while above 35° not a single specimen was found. Similarly, they were less numerous where the side of the fissure was cooler than 31°, while at 26° the few that occurred appeared less thrifty than under slightly warmer conditions. In areas still cooler than 26° the gametophytes were comparatively rare, but much larger sporelings had grown from them.

In another crevice under almost identical conditions, a small

part of the perpendicular wall protruded for several centimeters in the midst of a bed rich in gametophytes and minute sporelings. Immediately above this ridge all the sporelings were much larger, and had actively branched, but none of them extended beyond the protecting ledge into the rising steam. The general character of these plants, however, resembled that of the flora of cold, wind swept
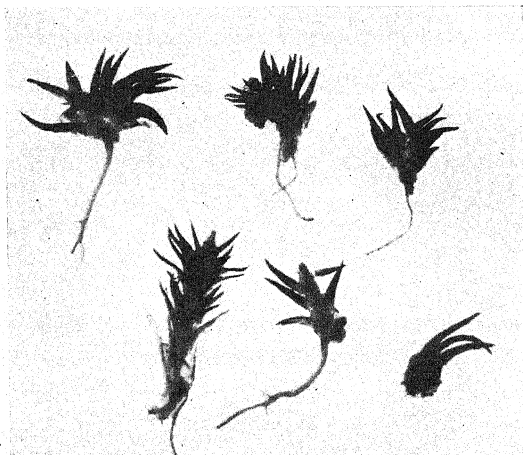


Fig. 2.—Sporelings of *L. cernuum* typical of warmer areas of volcanic steam crevices; ×6.3.

alpine regions. This same type of growth was observed in several other crevices where protection from the steam was similarly afforded. It may thus be inferred that the further growth of the sporelings in these fissures is inhibited by the hot vapor.

On a level rocky surface, in a spot that was covered by volcanic ash and unprotected from the sun, a small amount of vapor was noticed escaping from a crack 5 cm. long. On the leeward side and

hence exposed to steam, a tuft of moss was growing in which thrifty gametophytes and small sporelings were imbedded. From the position of these specimens it is plain that full exposure to sunlight is not detrimental. The reason why gametophytes in non-volcanic stations are so limited to shaded areas would therefore seem to be due, not to any ill effect of direct sunlight, but to the resultant desiccation of the surface soil to which they are confined. Stations are commonly situated on embankments, whether shaded or not, probably because such a vertical surface is not as likely to dry as is a horizontal one. Moisture that collects in the soil during rainy weather tends to seep out slowly. This not only keeps the side of the embankment moist during periods of drought, but insures good drainage during a wet season. On the other hand, a horizontal surface will more readily dry out during a short period of fair weather. That sunlight is not detrimental to the growth of the gametophyte and young sporeling, is further evidenced by the fact that the writer was able to keep typical plants growing for six months in direct sunlight without any noticeable ill effects. They were grown, however, under glass in an almost saturated atmosphere.

Pl. V at *X* shows a station of gametophytes and sporelings at the very brink of the crater of Kilauea, while the gradual slope of Mauna Loa is seen dimly in the distance. This station is unusual, yet it illustrates well the occurrence of the lycopods under heated conditions. In this case the steam escapes from the side of the precipice at no great distance from the plants. The soil upon which they are growing is heated mainly from below, but the entire plants at the edge of the crater are also frequently exposed to the hot vapor. Pl. VI is a closer view of the same plants. The match (*A*) nearest the brink of the precipice marks the spot where the thermometer registered 35° C. Here neither gametophytes nor sporelings were growing. Where the second match (*B*) stands, the temperature stood at a little more than 33°. Here were found many gametophytes and many minute sporelings; the latter are plainly visible in the photograph. The pencil (*C*) marks the position of a thick tangle of large sporelings. The thermometer here showed a temperature of 29°. The relationship between the temperature and the size of the sporelings is likewise exhibited in the typical crevices. Above approximately 35°

no lycopods can grow. At a slightly lower temperature they are extremely common but invariably small, usually little more than in the protophyll stage. At a still lower temperature almost all the sporelings are large, spindly, and much branched. Where the effect of the heat is negligible, the sporelings may be missing entirely, as is shown in this photograph. Or, if larger sporelings are present near the edge of the heated station, they appear not to have developed in that immediate locality from gametophytes, but rather to have spread by means of runners from the volcanic stations.

At the time that these photographs were taken (August 1923), these crevices were further investigated. In part of one crevice, from which a bed of moss several centimeters in thickness containing gametophytes and sporelings had been removed seven months before, several prothallia of different ferns were observed, among which were three gametophytes of *L. cernuum*. Two of them had sporelings with three protophylls attached; while from the third, a fourth leaf was just developing. If we exclude the possibility of minute gametophytes washing down to that spot from the moss above, it is practically certain that these three specimens developed to that stage within little more than half a year. This is not surprising in view of the occurrence, previously described, of specimens growing on the roadside that had been scraped by a passing vehicle.

Some of the crevices at that season were so dry that the mass of *Gloeocapsa* had dried into a firm gelatinous skin. Upon this many gametophytes were seen that varied from almost microscopical size to occasional specimens 7 mm. long and 5 mm. wide. From other heated localities were taken moderately dry layers of fine moss free from *Gloeocapsa;* here, in area of only 4 sq. cm., fourteen unconnected gametophytes and sporelings were gathered. In another clump of moss that was of looser texture, scores of minute gametophytes were gathered merely by tearing the moss to pieces in the field with a penknife. Most of these were about half a millimeter in diameter, and spaced 2–3 mm. apart. All appeared to be entirely green. They were situated at a depth of about 5 mm. in the moss where it had just turned brown. Whether the conditions for the development of the gametophyte are as favorable in the mass of *Gloeocapsa* is not known. Comparatively few gametophytes without

sporelings were collected with this blue-green alga, although the minute sporelings were extremely common.

In comparing these volcanic stations with the stations located under normal conditions, it might be thought that the great profusion of specimens in the former was entirely due to the moisture laden atmosphere. This, however, does not seem to be true. On the contrary, the presence of heat is the major factor for the prolific development of gametophytes and minute sporelings. Proof of this is afforded by the presence of the specimens in greatest numbers in the crevices where the substratum is heated to a certain definite range of temperature, while negative evidence is afforded by the uniform moisture conditions of the entire crevice and of its rim. The whole surface of such an area drips with moisture. The air is also saturated, so that in a few minutes one is covered with minute droplets of water. In some localities it is impossible to collect specimens because of the unbearable heat of the air, which, escaping from certain parts of some fissures, is blown by the wind from one place to another. If moisture were the only factor involved in this particular case, the entire area should have been covered uniformly with specimens. This by no means implies that volcanic heat is required by the gametophyte. So far as moisture is concerned, the conditions are probably the optimum, and are constant, but the specimens are not found in such profusion because of the moisture, but rather because of the exceptionally high temperature. In the non-volcanic stations, on the other hand, lack of moisture is probably the factor that kills off the gametophytes most commonly. We are justified in concluding, therefore, that the gametophyte and the minute sporophyte have an extraordinarily high optimum temperature and a very high optimum moisture requirement.

It is with design that the term "minute sporophyte" is used, since, as already stated, only small sporelings were found in such exposed volcanic stations. Wherever sporophytes above a few millimeters in length were found in the steam crevices, they were invariably protected from the greater part of the heated vapor. In spite of this protection these sporelings were commonly dying. Only such as were near the edge at the top of the crevices, and had been able to creep away from proximity to them, had continued their growth

and were developing into mature sporophytes. In spite of the great
number of gametophytes under heated conditions, the larger spore-
lings were far less common there than in non-volcanic stations, where
the gametophyte was rare. It should be remembered that around
both types of station *Gleichenia* was very common, and formed a
close association with the lycopod. Since in Goebel's (4) own words,
"die Gleichenien zu den an offenen, zeitweise trockenen Standorten
wachsenden Farnen gehören," we can apply the same remark equally
well to the mature sporophyte of *Lycopodium cernuum*.

These facts justify the conclusion that the older sporeling and
the mature sporophyte have an optimum temperature requirement
that is similar to that of the average flora of the region. They also
have a lower optimum moisture requirement and a very low mini-
mum moisture requirement, both considerably lower than the corre-
sponding moisture requirements of the gametophyte.

### Historical

These facts lead to certain interesting speculations, but before
considering them it will be well to refer to some of the literature
pertinent to gametophyte ecology.

An early report (1827–28) that has a bearing upon the present
problem is by Blume (2). He describes what might be considered a
variety or form of *L. cernuum* as *L. vulcanicum*. This plant, he
writes, "Crescit ad margines craterum montium ignivomorum
Javae." In 1841 Spring (10), in his monograph of the lycopods,
observes of *L. cernuum* that, "La localité des îles des Açores, sur le
parallèle de Lisbonne est la plus boréale de toutes celles qu'affecte
l' espèce. Le *L. cernuum* y a été trouvé, suivant une communication
que je dois à M. Gay, par Guthnik, voyageur des naturalistes à
Esslingen, autour d' une source chaude, sur un ancien volcan de
l' île de Pic." A similar report was made by Berkeley (1) in 1857.
He states that "A curious instance of the appearance of a tropical
species in temperate latitudes is afforded by *L. cernuum*, a species
very widely diffused in the tropics. It occurs about the warm springs
of the Azores in Terceira and St. Michael's, in spots exposed to the
sun, and again in the southern island of St. Paul, a fact which has its
parallel in the occurrence of a tropical *Pteris* under similar circum-

stances in a small island in the Mediterranean." To this is appended the following footnote: "The ticket which accompanies the specimens in the Hookerian Herbarium states that the temperature of the water was 114°, the air at the same time being at 65°." These temperatures in centigrade would read about 46° and 18°.

In 1884, TREUB (11) published his work on the sexual generation of *L. cernuum.* In spite of his exhaustive researches, he gives but a brief account of the type of locality in which the gametophyte of the species thrives. As is well known to those who have collected the sexual generation of plants of this genus, the mature sporophyte and the gametophyte of the non-epiphytic lycopods are rarely found in the same type of locality. It is therefore especially to be regretted that a fuller description of his stations was not included. TREUB sowed the spores of several lycopods upon tree trunks in the garden at Buitenzorg, Java, and met with considerable success in obtaining prothallia. He raised gametophytes of *L. cernuum* in his room, by sowing the spores on a kind of clay upon which he had noticed the prothallia growing in the natural stations. In a later publication (12) he mentions the fact that he was able to gather hundreds of gametophytes of *L. cernuum* in April. He attributes the abundance of these plants at that time to the excessively heavy rains, which, falling in the west of Java in January and February of that year, probably facilitated the germination of the spores. Not until his discussion (12) of the endophytic fungus does he allude to the type of station which he has found to be best adapted to the development of the gametophytes. There he gives the character of his stations in the following words:

Sans vouloir dire de ce Lycopode qu'il fuit les terreins riches en humus, il est certain qu'il croit de préférence sur un sol dépourvu de détritus végétal. J'ai récolté les prothalles et les plantules qui m'ont servi dans mes recherches, sur des terreins ou, le plus souvent, il paraissait ne pas y avoir de couche de humus du tout. Une espèce de terre glaise rougeâtre, fréquente dans les environs de Buitenzorg, est ici la station préférée par le *L. cernuum.*

In 1898 RACIBORSKI (9), speaking of *L. curvatum* Sw., and giving as the synonym the name "*L. vulcanicum* Blume Enum. 266; *L. curvatum* Sw. Spring, 81; *L. cernuum* var. *curvatum* Baker 23," reports it from Java as being "Ein Erdfarn des nicht stark beschat-

teten Waldbodens und der baumlosen Stellen der unteren mittleren und oberen Gebirgszone zwischen 1400–3000 M. Eine charakteristische Kraterflanze der Vulcane Java's, aber auch weit von den Kratern wachsend. Salak, Gedé, Pangerango, Telaga, Bodas, G. Guntur."

That same year KERKHOVEN (8) reported the species growing at about 1.5 km. and upward on the lava slopes of Guntur, one of the active volcanoes of western Java. *Gleichenia* is also reported as growing to man's height in the neighborhood of the crater.

In 1916 HERTER (5) mentions this lycopod as being found in the Bismarck Archipelago of German New Guinea, "In der Nähe der Solfataren, auf erhitztem Boden auf dem Vulkan Ghaie . . . . , in der Nähe von durch Schwefeldämpfe erhitzter Lava. Häufig und gesellig, charakteristisch an und um den Krater des Vulkans, selbst auf neuen Lavamassen, aus deren Spalten Schwefeldämpfe kommen." For New Britain he reports its occurrence as "Krater bei 'Die Mutter.' "

Not until 35 years after TREUB's discovery do we get another record of a find of gametophytes. It is to HOLLOWAY, who has worked upon the sexual generation of the New Zealand lycopods, that we must turn for data that seem to have most application to the ecological studies discussed in these pages. He states that *L. cernuum* "grows very abundantly throughout the northern part of the North Island of New Zealand on clay moorlands. . . . . It thrives especially in North Auckland amongst scrub vegetation of the *Gleichenia-Leptospermum* association, individual plants often attaining to a length of 12 to 15 ft., and the upright branches to a height of 1–4 ft. It is also extremely common on the Volcanic Plateau, in the neighborhood of hot water and near fumeroles" (6). He also states that *L. cernuum* is abundant on the clay gum lands of the Auckland Province, areas occupied at no great bygone period by the Kauri-tree forests, but "it shows still greater luxuriance in the neighborhood of hot-water streams."

On many occasions, and in many different parts of the Auckland Isthmus, as also in the Mongonui County, I have noticed young plants of this species growing in the vicinity of older plants. The spores would seem to germinate very freely. But . . . . a dry summer would bring about the destruction of most of

the plantlets. A damp clay bank or a shaded roadside cutting in the neighbourhood of adult plants is the best place to search for the young plants and prothalli —i.e., they are more in evidence under artificial than under natural conditions. Such a clay bank, if damp, shaded, and old enough for a thin covering of moss and slime fungus to have appeared on it, invariably contains the young plants of this species, generally in great abundance. . . . . The very young plants and prothalli of *L. cernuum* are difficult to clean owing to the intimate penetration of the clay and slime by their numerous rhizoids.

Later, HOLLOWAY (7) states that "They were all dissected out of humus consisting for the most part of a decaying short moss." In referring to the many species that he has collected, an additional statement (6) is worth quoting:

From my own observations, extended over a good number of years and in many parts of New Zealand, I have found it to be an almost invariable rule that young plants and prothalli are not to be met with in localities in which the adult plants are abundant. It is only in special localities, such as a damp shaded clay bank or roadside cutting, or some other patch of recently disturbed soil in the neighbourhood of adult plants, that the young plants occur. But it must be added that when favourable conditions are present prothalli occur often in the great abundance. . . . . Where the land-surface was deeply covered by volcanic ash during the eruption of Tarawera in 1886, *L. cernuum* was growing in abundance some years ago near certain streams of hot water. Colonies of this description must have originated from spores, but, unfortunately, no exact data are available as to the first appearance of young plants.

### Theoretical considerations

From a consideration of the stations discovered in Hawaii, and of the brief review of some of the localities from which this lycopod has been reported by other investigators, it is evident that three general types of stations occur. The gametophyte is found in two, and the sporophyte occurs in the third.

The type of station that must be the most common for the gametophyte throughout its range in the tropics is generally a moist clay embankment. Such an embankment is usually partly shaded, and often of comparatively recent origin, so that fine mosses predominate. A red clay composed largely of aluminum, iron, and silica seems to be preferred. This may be due to the fact that this lycopod requires large amounts of these elements, since its ash is composed of over 16 per cent aluminum and 30 per cent silica (3).

By far the most favorable station for the gametophyte, and the

minute sporeling that may arise from it, is limited to regions of volcanic activity. The substratum may be one of almost bare clay, or preferably a mat of fine mosses. This is frequently overgrown by a species of *Gloeocapsa*, which, if too thick, possibly tends to kill the lycopods. The latter are found imbedded in the moss or in the mass of blue-green algae. The atmosphere and the substratum are saturated with moisture and heated to approximately 31° C. The type of station especially adapted to the mature sporophyte, on the other hand, differs greatly from both types of stations adapted to the gametophyte. The sporophyte is found commonly in open glades and on the outskirts of forests, where it is frequently exposed to seasonal dry weather, so extreme as to be fatal to the gametophyte. It grows particularly well on volcanic soil, where it is often associated with *Gleichenia* in forming almost impenetrable thickets.

We are now in a better position to understand why the mature sporophyte of *L. cernuum* is not found in stations in which the gametophyte is common, and vice versa. It is because the mature sporophyte, as already explained, has an optimum temperature that is similar to that of the average vascular plant in its vicinity, and lower than the optimum temperature for the gametophyte. It has a lower optimum moisture requirement than the gametophyte, and, in particular, the capacity to endure a low minimum moisture condition. The conditions that make the locality particularly fit for the one stage of development, therefore, are quite unsuited to the other. It is for this reason that the gametophyte and the mature sporophyte are not found growing in the same habitat.

That this difference in the moisture requirements between the gametophyte and the thickly corticated sporophyte should obtain, is easily understood from a consideration of the delicate nature of the sexual generation, but why such a great difference in the heat optima should occur can only be conjectured. Nevertheless, the facts may justify a tentative suggestion.

If we accept the antithetic theory of the alternation of generations in plants, we must consider the gametophyte as the primal generation, from which the sporophyte has gradually developed by the intercalation of a post-sexual generation. Now if we take the two alternate generations as but one individual plant, which in a

certain sense it is, we are forced to concede that the gametophyte is the earlier part formed.

If there be any truth in the trite statement that ontogeny recapitulates phylogeny, we cannot consider this solely from a morphological viewpoint. It is the inherent qualities of the living protoplasm that function, or express themselves, according to a definite sequence, as formulated by this law; and it is the result of this progressive ontogenetic change in the protoplasm that is so easily perceived in the morphological changes. Since the latter is but the indirect effect, it is really the physiology that should be considered first in a study of the morphological ontogeny. If the physiology of a plant varies according to its stage of development, it is evident that the environment best suited to it must also be different. Thus if the plant is in an early stage of its development, the conditions under which it will grow best might well be similar to those to which its far-off ancestors were habitually exposed.

The striking structural contrast of gametophyte and sporophyte easily explains their very diverse habitats, as Bower has shown. The prothallus is almost hydrophytic, requiring a continuous and abundant supply of water, although living on land. It is so delicate that it cannot withstand the effect of dry conditions for any length of time. It has neither roots for the absorption of water from below the surface of the soil, nor a vascular system to conduct this water through its tissues. Furthermore, it is absolutely dependent upon water for the liberation of its sperms and the fertilization of its eggs. The morphology of the sporophyte, on the contrary, points to an almost xerophytic habitat. It has a very thick cortex that inhibits the transpiration of moisture; it possesses roots for absorption and a complex vascular structure to aid in the conduction of water through its body; and it is dependent upon dry conditions for the liberation and dispersal of its spores. But are we not in reality considering the effect instead of the cause? It were better to state that the filament growing from the spore is limited, not adapted, to conditions of moisture because of its inherent physiological qualities. Its progenitors, of a type that were comparable with this stage, were presumably threadlike forms. The thalloid gametophyte, now somewhat altered because of the symbiotic fungus, is limited to a semi-

aquatic habitat, because its phylogenetically comparable ancestors grew under similar conditions. What is then more likely than that the minute sporeling just recently developed from the gametophyte should also be limited to conditions little different from those under which the sexual generation thrives best?

If, instead of the antithetic, we advert to the homologous theory of the alternation of generations, this difference in the optima for growth in the different stages of development might be explained as follows. If the two generations were distinct from the beginning, the asexual stage might have been the more plastic; it could, therefore, in later times adapt itself far more readily than the gametophyte to the more prevalent ecological conditions.

The great profusion of gametophytes and small sporelings under conditions of abnormal heat and saturated atmosphere may then be due to the following fact. The conditions which are the optimum for the gametophyte and minute sporeling simulate the climate of that geologic period in which the progenitors (that never advanced ontogenetically beyond such a general state of development) found their optimum conditions for growth. On the other hand, the older sporophyte has diverged too far in its environmental requirements from the gametophyte to be able to grow successfully in a situation that is suited to the latter. The conditions that are necessary for the one stage have gradually become inimical to the other.

Should this divergence in environmental requirements between the gametophyte and sporophyte generations be true, we should be able to find similar cases among plants that stand on a similar level of phylogenetic development. A few instances which apparently support such a theory have come to attention.

About 75 m. distant from the *Lycopodium* stations, and on the other side of the perpetual firepit called Halemaumau, a long crevice about 2 m. in width and 3 m. in depth was found. This was situated near the Kau Desert, in the midst of bare lava, much of which had flowed to that vicinity not many years before. The bottom of this crevice had been filled in by natural means, so that it was possible to walk upon it. Scarcely any steam could escape, although the ground was intensely hot.

The side of the crevice showed stratification, the rock being

interbedded with sandy layers of ash. All was heated volcanically and was quite moist, although not saturated. In many places a whitish salt incrusted the surface. Upon one side of the crevice, a great profusion of *Psilotum triquetrum* Sw. and *Ophioglossum* sp. was growing. The former was the more common plant, and very often grew between the cracks in the rock layers; the latter was limited to the sandy layers only. Because of the isolation of this crevice in the midst of a desert of lava, there is little doubt that these plants developed in this situation by spore dispersal. Also, because of the great number of individual plants of *Psilotum* growing in isolated cracks in the rock, it is probable that this method of propagation was frequent. That the same was also true for *Ophioglossum* is probable, since many plants were growing in the layers of decomposing ash, isolated one from the other by the rock strata. Although *P. triquetrum* is very common in this general locality, and is adapted to the "*a-a*," or rough lava fields, the *Ophioglossum* sp. apparently is quite rare. Throughout the entire time that the writer collected plants in this region, only one other specimen was found. It was discovered the same day about 1.5 km. away, under an overhanging bank in a deep gully in the Kau Desert. The only other plant growing with this specimen was a species of *Marchantia*.

Although one cannot make a true comparison of these stations based upon plants belonging to different orders, yet since these plants possess certain characteristics in common, and appear to be the remnant of an archaic flora, a comparison is worth attempting. It will be noted that in the Kau station the conditions of the substratum, so far as heat is concerned, were practically identical with those of the volcanic lycopod station. The moisture content was also similar. In both stations the sexual generation appeared to have developed under unusually favorable conditions. Where the conditions were those of heat and moisture, the sporophyte of *Lycopodium* failed to develop beyond its earliest stages; while where the conditions of unusual heat and moisture were absent from the air above the Kau station, the sporophytes of *Psilotum* and *Ophioglossum* developed in a normal manner.

It must be remarked that neither specimens of *Psilotum* nor *Ophioglossum* were found in the volcanic lycopod station. In fact,

*L. cernuum* was the only vascular plant present. The subterranean nature of the gametophytes of these two plants may account for this absence. A saturated substratum, as found at the lycopod station, would prevent every access of air to them and quickly cause their death. The prothallium of *L. cernuum*, however, being at the surface of the ground, is always exposed to the air or in close proximity to it, no matter how saturated the substratum may be.

That not a single lycopod was found in the Kau station was surprising. One explanation is that since it is limited to growing at the surface of the soil, the conditions at this station were too dry. With a sandy substratum, exposed to the sun, and with no water vapor to supply moisture, this plant would readily dry out. Another possible reason for its absence may be the presence of salts at the surface of the soil. That the subterranean gametophytes of the other cryptogams would neither be exposed to drying, nor to that concentration of salts at the surface, probably accounts for their presence in this station.

That the sporophyte and gametophyte of the more northern lycopods should also be found in stations by themselves, would tend to weaken the view that a heated environment more nearly approaches that to which the ancestral plants were accustomed. Yet we should consider that the gametophyte of *L. cernuum*, not excepting that of *L. Selago*, is the most primitive type known for the genus. Nor would this instance militate against the theory of divergence in respect to other environmental conditions. It is a fact, however, that in its ontogeny the sporophyte becomes more and more adapted to an environment that is unfavorable, if not fatal, to the gametophyte from which it develops.

This very inadequate study may give the clue to a vastly greater problem, one upon which BOWER has fully touched in his writings. From about Middle Devonian time, cryptogams with a well defined sexual and asexual generation became common. Such plants then constituted the predominant land flora of the Late Paleozoic or Carboniferous, but by the time of the Mesozoic, practically all the more significant spore bearing plants of the former era were gone. The others, the ferns excepted, have gradually become

extinct, until but a few insignificant representatives of this archaic flora remain to the present day. It may be suggested that with the changing environmental conditions during the latter part of the Carboniferous, those plants that were the least plastic, from a physiological point of view, quickly disappeared. Those remaining tended to adapt themselves to existence on a drier terrain. In most cases one generation was unable to change its requirements for growth as quickly as the other. This resulted in a progressively greater divergence in their respective optima for existence. The sexual generation, however, being built upon a plan that is dependent upon moisture, found it more difficult to become adapted to a drier climate than the asexual generation, which, from its beginning, had been dependent upon relatively dry conditions for its chief function of spore dispersal. Thus we find that by the beginning of the Triassic, those plants that were still keeping in the race between their minimum requirements for existence in the sporophyte generation and the ever changing conditions of their environment, had picked up their lagging gametophyte generation. They carried it from the ground where it could no longer surmount the obstacles in the environment, and protected it within sporophytic tissues where a suitable artificially created environment was possible. Thus, in order to survive, the sporophyte generation had given protection to the gametophyte generation. Henceforth the latter became more and more dependent and progressively reduced, until in the present era the dominant angiosperms harbor barely more than the essential reproductive organs of the gametophyte. Of course, the foundation for these theoretical considerations is a very slight one; at least it may furnish a basis for further investigation.

## Summary

1. On the island of Hawaii, and under normal ecological conditions, six stations for the gametophytes and small sporelings of *Lycopodium cernuum* L. were discovered; on the island of Oahu and under similar conditions another station was found.

2. On the sides of volcanic crevices near the crater of Kilauea, and under conditions of abnormal heat, thousands of gametophytes and small sporelings of *L. cernuum* were discovered.

3. The gametophyte of *L. cernuum* grows far better under volcanic conditions than under conditions without volcanic heat.

4. Exceptionally high temperature and abundant moisture are two of the optimum conditions for growth of the gametophyte of *L. cernuum*.

5. The conditions of heat and moisture that are best suited to the development of the gametophyte of *L. cernuum* are injurious to the development of the sporophyte.

6. Through a tentative theory of divergence, an attempt is made to account for the difference in environmental optima of the two generations.

7. Such a theory of divergence may explain in part the gradual extinction of the cryptogamic flora of past geologic eras.

This paper was begun at the University of Hawaii, in partial fulfilment of the requirements for the degree of Master of Science. The writer wishes to express his great indebtedness for encouragement and advice in the preparation of the manuscript chiefly to Dr. JAMES B. POLLOCK, former Exchange Professor from the University of Michigan at the University of Hawaii. For similar favors thanks are due to Dr. HAROLD S. PALMER of the latter institution, as well as to Professor A. VINCENT OSMUN of the Massachusetts Agricultural College. Through the kindness of Dr. T. A. JAGGAR, Director of the Hawaiian Volcano Observatory, it was possible to illustrate the paper with photographs of the stations.

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PLATE IV



DEGENER on LYCOPODIUM

PLATE VI

PLATE VII
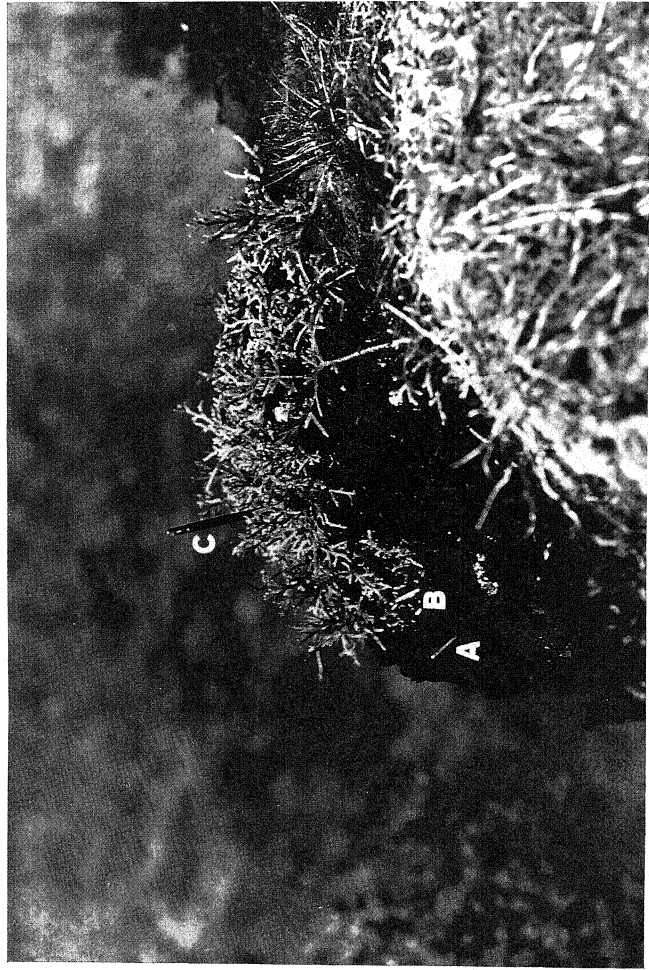
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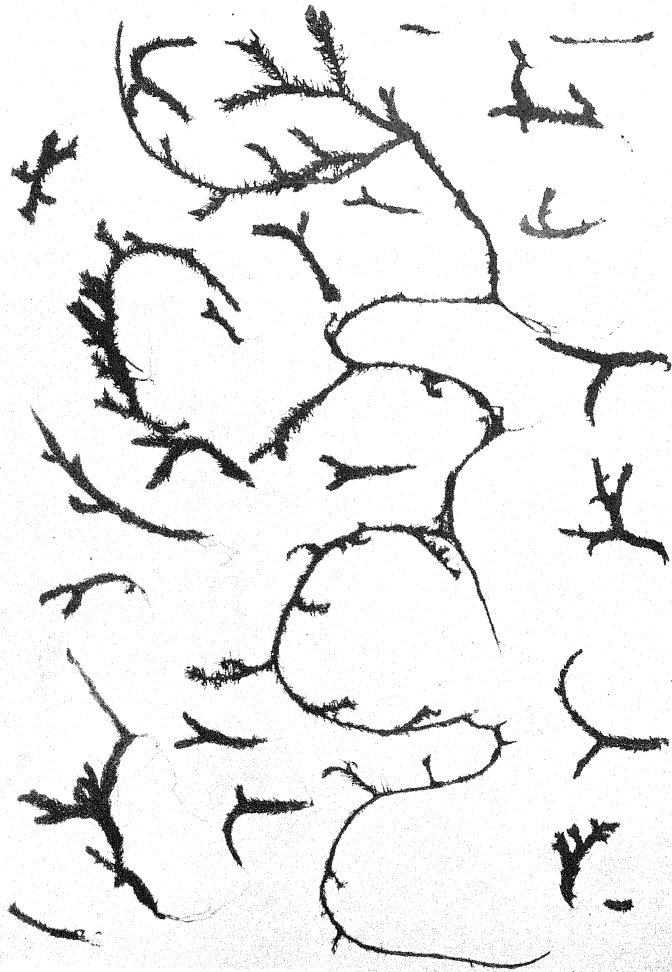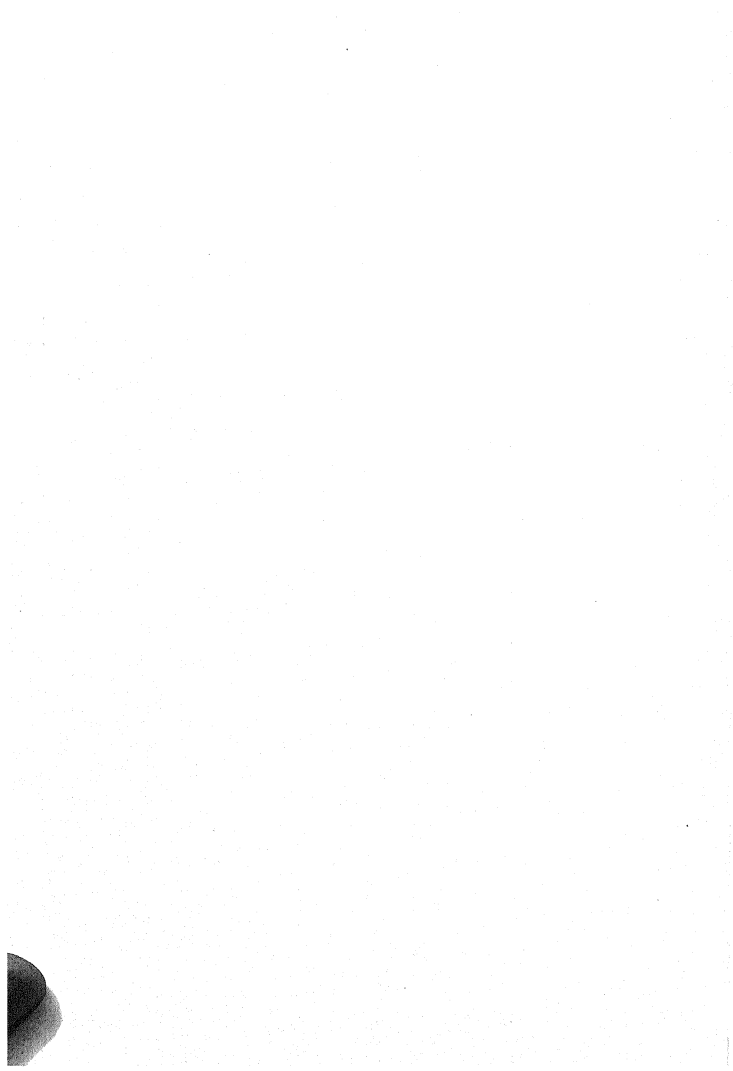
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## EXPLANATION OF PLATES IV–VII

PL. IV.— Volcanic steam crevices in which grow thousands of gametophytes and sporelings of *Lycopodium cernuum*.

PL. V.— Station of gametophytes and sporelings of *L. cernuum* (at *X*) growing at brink of crater of Kilauea under abnormal conditions of heat and moisture, due to proximity to steam vent.

PL. VI.— Closer view of some lycopod station: at *A*, where lycopods are absent, thermometer registered 35°; at *B*, where gametophytes and only small sporelings are growing, thermometer registered about 33°; at *C*, where large much branched sporelings are very common and gametophytes very rare, thermometer registered 29° C.

PL. VII.— Larger sporelings of *L. cernuum* from stations in volcanic steam crevices or at their very brink (with exception of largest plant).

# STRUCTURE OF THE RHIZOME OF
## EQUISETUM GIGANTEUM

### ISABEL M. P. BROWNE

#### (WITH THREE FIGURES)

### Introduction

The fragment of the rhizome of *Equisetum giganteum* L. on which the present observations were made was supplied by Professor R. C. MACLEAN of Cardiff, to whom I wish to offer my best thanks. The material consisted of a single node of the rhizome with considerable portions of the internodes above and below. The exterior of the rhizome was bluntly and obscurely angled, but there were no obvious ribs. The hardness of the external layer of the rhizome made the preparation of serial sections very troublesome, especially in the region of the node, and I was unable to avoid local tearing of the tissues.

The diameter of the axis in the internodes is about 5.5 mm., and that of the central cavity about 2.5 mm. At the organically lower end of the specimen the axis contains nine bundles and vallecular canals. Each bundle possesses a distinct endodermis or sheath. There is no general endodermis. In the internodal condition the bundles are separated by spaces about two and one-half times their own size. The outline of the bundle is elliptical, the longer axis being directed radially. The carinal canal is large, occupying about half the bundle; its greatest width may be in a radial or in a transverse direction. Usually hardly any remains of the protoxylem are to be seen in the carinal canals. The phloem forms an arc, slightly concave internally, at the periphery of the bundle. The lateral groups of tracheids sometimes abut on the ends of the arc of phloem, or they may be somewhat more deeply seated. The space between the arc of phloem, the lateral groups of metaxylem, and the carinal canal is occupied by parenchyma, of which the middle cells are relatively large, thin walled, and slightly elongated radially. Each lateral group of metaxylem generally contains four to six, sometimes only

two or three reticulately thickened tracheids. More often than not
the middle tracheids of a group or band appear to be the largest, but
there is no regularity in this. The tracheids may be arranged in a
more or less regular band, usually uniseriate; or they may be dis-
posed in a group in which there are locally two or three tracheids
side by side. The vallecular canals are large and bluntly triangular.
They are separated from one another by radiating bands of paren-
chyma, four to eight cells in thickness, which lie opposite the bun-
dles. The blunt apices of the triangular vallecular canals run in
between the bundles, reaching to about the depth of the outer limits
of the carinal canals. They are separated from the carinal cavity
by about four to eight rows of cells. The cortical tissue between the
bundles and the radiating bars of cortical parenchyma separating
the vallecular canals contains numerous starch grains, while the
outer layers of the cortex consist of small cells, either devoid of or with
very few starch grains. The outer layer of the rhizome possesses a
thick brown cuticle, and its cells have dark, thick walls. The next
layer consists of smaller cells, the walls of which are also strongly
sclerized. This marked thickening extends to a third layer of cells,
especially to their outer walls. Altogether there are six to nine rows
of cortical cells between the epidermis and the vallecular canals. The
cells gradually become larger and thinner walled toward the interior.

## Nodal structure

As we pass organically upward in the series of sections through
the relatively main rhizome, indications of the insertion of the stele
of the branch begin to appear. As the branches are inserted between
the bundles of the internodes below them, the outer and lower por-
tion of the obliquely inserted ramular siphonostele is inserted on two
lateral groups of metaxylem belonging to different bundles. It is
noteworthy that the tracheids of the lateral groups of metaxylem
on which the ramular siphonostele is inserted higher up assume
nodal character before the tracheids of their fellow groups in the
same bundle. GWYNNE-VAUGHAN (3) showed that in the aerial
stems all the tracheids of the metaxylem have reticulately thickened
walls, and this is true of the metaxylem of the rhizome. In the latter
the elongated narrow metaxylem tracheids are gradually replaced,

as the node is approached, by more numerous short, wide tracheids of
the usual nodal type and with a different kind of reticulum.

One of the preparations for the development of the branch is the
division of the vallecular canal opposite into two by a somewhat
wide band of parenchyma. The vallecular canals are so wide (at
their widest more than 1 mm. across) that two small canals remain
on either side of the bridge, even while the latter is being traversed
by the stele of the branch. The ramular stele is nearly 0.5 mm. in
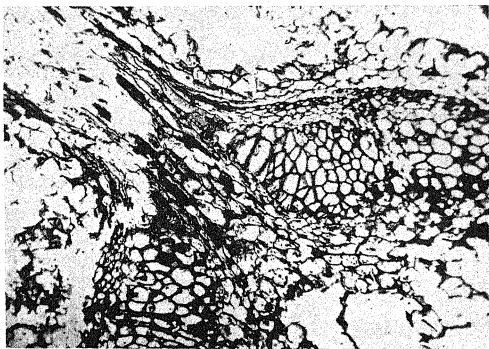


FIG. 1.—Transverse section of part of node of rhizome of *E. giganteum*, showing
insertion of ramular stele; note narrow elongated tracheids (protoxylem?) inside ordi-
nary nodal ones; near either edge may be seen xylem of leaf trace, one or two nar-
row elements, passing through nodal xylem.

diameter, and its xylem consists entirely of reticulately thickened
tracheids. Most of these are of the usual nodal type. Some of
the more internally situated tracheids, however, offer a strong
contrast to the others. They are narrow, relatively elongated ele-
ments, with thin reticulations (fig. 1). It is possible that they repre-
sent protoxylem of a modified sort, for, as will be explained later,
the axial protoxylem of the rhizome consists of somewhat similar
elements. Such a differentiation between the tracheids composing
the xylem of the siphonostele found at the base of all the branches

throughout the genus does not seem to have been recorded before, and does not occur in the steles of the branches of the aerial stem (BROWNE 2). These narrower tracheids belong definitely to the stele of the branch, and are not in connection with the axial proto-xylem.

Three branches were initiated at the node in question. None of these had broken through the sheath of the parent axis; all of them had reached much the same stage of development. The vascular elements below the first diaphragm of the branch seemed to be fully differentiated. The tracheids were fully lignified, and in the pith below the diaphragm was a central group of dark brown cells. Although the diaphragm itself is already clearly indicated as consisting of several rows of short cells, the tissue above it is in a highly immature condition. Above this level no indications of vascular tissue can be distinguished, but the branches, which had hitherto grown out nearly at right angles to the parent axis, turned obliquely upward toward the organic apex of the latter. A little higher up in the series of sections the ochreola, or first and rudimentary leaf sheath of the branch, may be distinguished abaxially and laterally as a sheath two cells in width, just becoming free from the axis (fig. 3). Like the ramular siphonostele, the ochreola is inserted obliquely, the side away from the main axis being lower than the adaxial side. On the branches in question the ochreola was not yet discernible on the adaxial side.

All the branches gave rise to a pair of rootlets. These arose at opposite points on the sides of the branch, one root being at its insertion directed toward the organic apex of the main rhizome, and the other in the opposite direction. Their vascular supply is given off at exactly opposite points, is equal in amount, and has attained a similar degree of differentiation. In fact, everything points to the two roots of a branch being equivalent and contemporaneous in development.

Both roots eventually grow backward along the rhizome, that is, away from the organic apex of the main axis. Thus the steles of both roots, each with a sclerenchymatous sheath, appear obliquely cut at the edge of the sections through the main rhizome, before the insertion of the branches is reached. They are here seen passing

through tissue which has been shown in other species to belong to the leaf sheath. This tissue has become concrescent during ontogeny with the tissue of the main axis. So intimate is the concrescence that all boundaries between the two tissues have been obliterated, except at the point of separation of the leaf sheath from the axis. Here the junction of the free epidermis of the axis with that of the upper surface of the leaf sheath is marked by a double band of sclerized cells.

These dormant branches, or rather their apices, lie in the space between the parent axis and its leaf sheath. The concrescence between these two is continued longer opposite to the leaf traces, and these only pass out into the sheath at a level at which the branch apex is lying between the sheath and the main axis and has assumed a direction almost parallel to that of the latter.

In bundles preparing to give off traces the carinal canals usually become filled by a non-cellular substance staining deeply with Vesuvian brown, presumably by mucilage. In this substance may be seen the remains of tracheids of rather various sizes, adherent to the walls of the canal. Eventually, before the leaf traces are given off, the carinal canals disappear and their place is taken by short, wide, reticulately thickened tracheids of the nodal type, somewhat smaller than the more peripheral ones. Interspersed among these more internal nodal tracheids, which first make their appearance a little higher up than the other nodal tracheids, are two other kinds of elements. Firstly, there are occasional parenchymatous cells of much the same outline as the tracheids between which they lie. Intermediate between these parenchymatous cells and the ordinary nodal tracheids are elements in which the wall is already partially lignified, but the cell contents, although contracted, have not yet disappeared. Secondly, there are more numerous elements, also of the same shape as the tracheids, the lumen of which appears to be almost or completely obliterated, the cell contents being replaced by an opaque substance staining darkly with Vesuvian brown. At first sight these dark brown cells suggest small canals filled with mucilage, but by careful focusing the original tracheidal wall, stained by gentian violet, can be distinguished. If the same cell be followed through successive serial sections it can usually be seen

that a small central part of it is free from the brown substance. The
latter appears to adhere to the wall, and, moreover, stains rather
more deeply than mucilage. It is probably a kind of sclerized thick-
ening, although no stratification is visible, at least in sections 14 $\mu$
in thickness.

At the inner edge of the nodal tracheids, or separated from them
by one or two parenchymatous cells, are a few small elements of
protoxylem. When these are cut slightly obliquely their walls
appear to be reticulately and not spirally thickened. The bars of
thickening are thin, however, and do not fork freely, so that in a
longitudinal section of a tracheid the type of thickening is not very
unlike the spiral form usually found in protoxylem. A few of the
protoxylem elements in a median position pass through the axial
bundle, to depart as the xylem of the trace. The reticulate thicken-
ing of these departing tracheids, of which there are usually two to
four (never more than five), is well seen, since their long axes are
directed obliquely outward. The traces remain minute throughout
their course; nevertheless they appear to become concentric while
in the cortex. After the trace has become free from the bundle it
passes out very slowly, pursuing a steeply oblique course. As already
mentioned, the portions of the axis opposite to the traces remain con-
crescent with the leaf sheath, after the latter has become free from
the rest of its surface. Consequently the traces pass across paren-
chymatous bridges, and only enter the free portion of the leaf sheath
rather more than half a millimeter above the level at which they
detach themselves from the axial bundles. As soon as they have
passed over the sheath becomes completely free from the axis. In
the aerial stem the xylem of the trace was surrounded by a narrow
parenchymatous sheath while it was passing through the bundle (2).
In the rhizome there is no such definite sheath, but parenchyma-
tous cells, some of which have the same orientation as the depart-
ing tracheids, are relatively common at the level of departure of
the traces.

The vertical extent of the nodal xylem is much less than in the
aerial stem previously described (2). In the sectors of the stele
on which the ramular siphonosteles were inserted higher up,
tracheids of a nodal character make their appearance relatively

early; that is, they usually make their appearance at from 440 μ
to 640 μ below the level at which the dying out of the nodal
or supranodal tracheids, lying vertically above traces that have
departed, caused the reconstitution of the bundles in the upper
internode. In one case, however, the distance between these two
levels was only about 320 μ. In bundles on which no branch steles
were inserted, the nodal tracheids usually made their appearance
about 280 μ to 350 μ below the dying out of the nodal wood opposite
a trace that had departed, although in one such bundle some nodal
tracheids appeared as much as 420 μ below the level of the reconstitu-
tion of the bundles in the next internode. A few nodal tracheids
persist a short time in the reconstituted bundles, dying out about
230 μ to 280 μ higher up. It must be remembered that, as is so often
the case in *Equisetum*, the node is slightly oblique, so that there is no
continuous ring of nodal xylem to be seen in any section. Indeed,
two of the bundles failed to become connected by nodal wood, and
remained throughout the node separated by a narrow tract of
parenchyma. A similar example of reduction of nodal xylem has
been observed in cone-bearing branches of *E. debile* Roxb. (1).

Not only is the nodal wood of the rhizome much reduced in
quantity, compared with that of the aerial stem, but there are also
indications that it develops rather late. The rhizome studied was
fully mature, as is shown by the large size of the central, vallecular,
and carinal cavities and by the considerable length of the internode.
Yet the nodal wood contained a considerable number of undifferen-
tiated or incompletely differentiated elements.

Another indication of reduction of the vascular system at the
nodes is afforded by the discontinuity of the protoxylem in this re-
gion. Some axial protoxylem persists after the departure of the
trace, but such elements, which are very small and somewhat
crushed by the growth of the larger nodal tracheids, are usually
scattered. In some cases the bundles, as at first reconstituted, ap-
peared to contain a few elements of protoxylem continuous with those
of the internode below, but usually the protoxylem died out before
the formation of the separate bundles of the next internode. In any
case, the reconstituted bundles soon assume a more or less circular
outline, and it can then be seen that the larger tracheids fill up the

whole of the space included within the bundle sheath, except that occupied by the external arc of phloem. Eventually the bundle assumes the typical internodal form, the larger tracheids being replaced by parenchyma, except where they are replaced by the smaller tracheids composing the lateral groups of metaxylem. The more deeply seated large tracheids persist longer than the more peripheral ones. Protoxylem also reappears.

As compared with the aerial stem, the reduction of the xylem of the traces is much greater than that of the axis. In the aerial stems the trace contains, in addition to numerous elements of protoxylem, a considerable number of metaxylem tracheids. In the rhizome the traces possess no metaxylem, while the protoxylem may be reduced to two, and seldom consists of more than four elements. In the only specimen available, all the tracheids of the internodal lateral strands were completely differentiated. As the position within the strands of the smaller tracheids was variable, it was impossible even to conjecture whether the internodal xylem of these strands developed centripetally, as in the aerial stems, or centrifugally, as in the other axes, in which the development of the lateral strands has been followed.

### Root bearing branches of rhizome

JANCZEWSKI (4) studied the development of the root bearing branches of the rhizome of E. arvense and E. limosum, and came to the conclusion that true rhizogenous branches only occurred in the latter species. He stated that the branches of E. arvense became dormant after giving rise to one (or at most two) roots, and he noted that where there were two roots the second was inserted above the first, on the lower side of the branch with reference to the organic apex of the parent axis. He seems, in fact, to have held the modern view that these root bearing structures of E. arvense are merely arrested branches. In E. limosum, however, he distinguished two sorts of buds: a small number, usually not more than one at a node, of relatively voluminous branch buds, destined to develop as aerial stems or branches of the rhizome, and a larger number of true rhizogenous buds. He admitted that the mother cells of the branch buds and those of the rhizogenous buds had the same morphological value,

but he held that if, in spite of the difference in constitution of the
two kinds of bud, an attempt were made to bring the one type into
line with the other ("si . . . . on s'éfforçait de rattacher les bour-
geons rhizogènes au type des bourgeons à rameaux"), it would be
necessary to admit an analogy, physiological rather than morpho-
logical, between the rhizogenous buds and the basal internode of
the branch buds, and to suppose that in the rhizogenous buds the
vegetative apex was completely abortive. JANCZEWSKI further noted
that if the rhizogenous bud of *E. limosum* produced two roots, these
were inserted on opposite sides of the branch, one toward and one
away from the organic apex of the main branch. Where there were
four such roots they formed an irregular quadrangle. From the
state of development of the roots JANCZEWSKI concluded that the
first root to develop was that on the lower side of the branch, and
the second that opposite to this, the roots between them being of
later origin. Nevertheless, both his figures of young rhizogenous
buds show a pair of very young roots in essentially the same stage
of development.

More recently, JEFFREY (5), in treating of the rhizome of
*Equisetum*, also distinguished between nodal buds which develop as
rhizophoric organs and the less frequent ramular buds. I doubt
whether the distinction drawn by these investigators is a valid one.
For instance, JEFFREY, treating of the rhizome of *E. silvaticum*,
states that in the section of it figured by him the central cylinder
gives off six processes, five of which are root bases and one a dormant
branch. He further points out that on one side of this section the leaf
traces have entirely cleared the central cylinder, and an examination
of his photograph of the rhizome shows that it is between the two
traces that are farthest apart that his branch bud is found. It is
certainly suggestive that in serial sections through the node of the
rhizome of *E. giganteum* the three branches had, at a certain level,
much the appearance of JEFFREY's root bases or rhizophoric organs,
while slightly higher up they resembled his branch buds. If JEF-
FREY's section, instead of being itself slightly oblique or of passing
through a slightly oblique node, had passed through a region in
which the traces in the upper part of the photograph were cut as far
out in the cortex as those in its lower part, the rhizogenous buds

might have shown many of the same features as the branch buds.
A correct conception of the form of the dormant rhizogenous buds
can only be obtained from the study of serial sections, the thickness
and orientation of which are known with accuracy. Such sections
can hardly be obtained except with a microtome. In *E. giganteum*
the dormant branch turns sharply upward toward the organic apex
of the main rhizome, immediately above the first ramular dia-
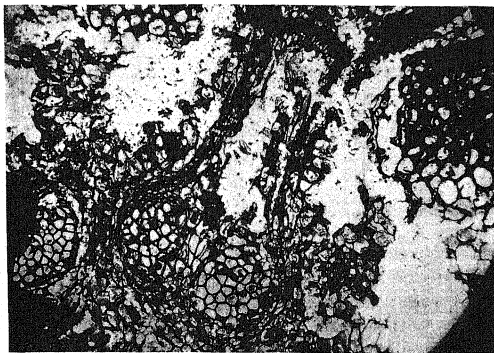


Fig. 2.—Transverse section cut slightly higher than fig. 1, showing one of bundles
just reconstituted, and at periphery the insertion of two equivalent root steles at level
of first diaphragm of branch; note occasional presence among tracheids of elements
with lumen partially or completely obliterated; ×50.

phragm. Also, in transverse sections of the main axis, which show a
radial longitudinal view of the ramular stele at its insertion on the
central cylinder of the parent axis, the branch appears to terminate
bluntly with the first diaphragm (fig. 2). At or just below this the
root steles may be seen departing from opposite sides of the ramular
stele. Only the study of the following sections reveals the existence
of a distinct vegetative apex, with, slightly higher up, indications
of the ochreola (fig. 3). JEFFREY's processes given off by the central
cylinder may well represent the outer, lower edges of the obliquely
inserted ramular siphonostele which they closely resemble. Simi-

larly, JANCZEWSKI's two figures of very young stages of rhizogenous
buds of *E. limosum* show a stage which is much like what the similar
stage, that before the development of vascular tissue, must have
been in the root bearing branches of the node of the rhizome of
*E. giganteum*. In fig. 2 of JANCZEWSKI's pl. II in particular, the
initiation of the roots can clearly be seen, while the branch appears
to terminate without a vegetative apex, just as it does in the more
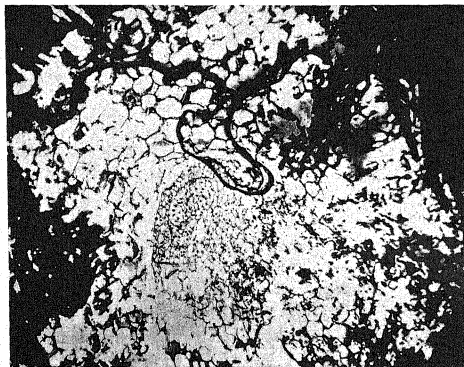


FIG. 3.—Oblique section of apex of branch above level of figs. 1 and 2; note
young ochreola; toward periphery is seen part of sclerized sheath of root bearing
branches; ×56.

advanced rhizogenous branches of *E. giganteum*, the apices of which
lie in a higher plane. The same thing applies to the diagrammatic
figure of a more advanced branch shown in JANCZEWSKI's fig. 5 of
pl. II. In this connection it is significant that, although JANCZEWSKI
stated that as a rule the mother cell of a rhizogenous branch does not
become or give rise to an apical cell, he added that the arrangement
of the cells in the young bud was sometimes such that the pre-exist-
ence of such a growing point might be presumed. He held that in
such cases the apical cell early underwent extinction by dividing up

in the same way as its first segments. Although the analogy with
the rhizogenous buds of *E. giganteum* suggests that a growing point
may well be present in the similar organs of *E. limosum,* an abortion
of this growing point in certain rhizogenous branches is not im-
possible of course. JEFFREY's researches show that a single dormant
branch of *E. limosum* may give rise to as many as six roots, and it
is not improbable that with greater root development the axial
part of the branch might become further reduced, and its apex
abortive.

### Increase of bundles in rhizome

As already stated, the lowest portion of the rhizome preserved
is internodal, and contains nine bundles. The upper internode, al-
though of much the same diameter, contains eleven bundles. One of
these fresh bundles was formed by the breaking of the nodal xylem
between the breaks formed opposite to two traces that had departed.
In this region the two traces were relatively far apart. There was
over the whole circumference of the stele a tendency for the traces
to arise at slightly unequal distances from one another, and by no
means always exactly opposite to the parenchymatous bridges which
they have to traverse in order to enter the sheath. It is curious that
it was not the traces on either side of the rather bulky rhizogenous
branches which were relatively far apart.

The other new bundle arose in the upper part of the lower inter-
node, between two bundles that were unusually far apart. At its
first origin it was quite free from either of its neighbors, although
much nearer to one of them than any of the other bundles were to
the others. The first indication of the new bundle is the appearance
of a single large tracheid, which is obviously of the nature of meta-
xylem. It is separated from the bundle nearest to it by about the
width of an ordinary internodal bundle, and is situated at about the
depth of the outer limit of the carinal canals. A little higher up
other somewhat smaller tracheids, also obviously belonging to the
metaxylem, make their appearance on the side toward the nearest
bundle. The new bundle is then represented by a a nearly trans-
verse band of metaxylem. The tracheids do not at first increase
rapidly and the band remains uniseriate. A little more than a milli-
meter above the first appearance of the large tracheid referred to

there were but five to seven tracheids. At this level the only other
indications of fascicular development is the presence of a tract of
parenchyma, approximately equal in size to the bundle, a tract of
tissue which would normally have been destroyed by the develop-
ment of a vallecular canal. A little higher up the number of tracheids
begins to increase rather more rapidly, and some of them are more
deeply seated, although they still obviously belong to the meta-
xylem. Soon there are eleven or twelve of these, as well as a single
very large, more internally situated tracheid. Thus the metaxylem
found at this level, although differently disposed, is about equal in
amount to that present in other bundles. Somewhat higher up the
new strand contains about twenty of these tracheids, and in places
the metaxylem may be as much as three elements in depth. The
cells surrounding these tracheids have remained smaller than the
other cortical cells, but have undergone no special differentiation,
so that there is neither phloem nor protoxylem in the new strand. It
is only about 3 mm. above the appearance of the first tracheid that
one or two cells of protophloem make their appearance. As does the
metaxylem, the phloem arises first on the side of the new bundle
away from the nearest neighboring bundle, and its subsequently
developed elements arise on the side toward this bundle. Just before
the appearance of the phloem the new strand has become inclosed
in a common bundle sheath with its nearest neighbor. About 350 $\mu$
above the appearance of the first element of phloem, a very large
tracheid, about three times the size of the larger of the other meta-
xylem tracheids, develops on one side of the new strand, in a position
markedly internal to the other tracheids, from which it is separated
by five or six cells. The first indications of protoxylem appear above
this tracheid. The protoxylem is represented by a small canal with
the remains of three or four tracheids in it. This canal lies at about
the same depth as did the large tracheid just mentioned, but it is
more centrally situated with reference to the new bundle as a whole.
The protoxylem canal widens rather rapidly. When the protoxylem
makes its appearance, the tracheids in the middle of the band or
group of protoxylem are replaced by parenchyma, so that the new
bundle, although smaller, is similarly constituted to the others. It
remains exceptionally close to one of its neighbors. One of the

three branches found at the node is inserted partly on the new bundle and partly on its more distant neighbor. The new bundle gives rise to a normal trace, so that there are ten of these at the node.

## Summary

1. In the only specimen of the rhizome of *E. giganteum* in which the internal structure has been studied, each of the bundles of the internode is surrounded by a special sheath. There is no common endodermis.

2. All the tracheids, those of the protoxylem as well as those of the metaxylem, have their walls reticulately thickened.

3. The protoxylem is discontinuous at the nodes.

4. The basal region of the ramular siphonostele, that is, that below the first diaphragm of the branch, shows a certain differentiation of the xylem. Most of the tracheids are of the usual nodal type: short, wide, and with numerous reticulations. At the inner edge of the stele, however, there are in places a few much narrower, elongated elements with a thinner reticulum. These are not continuous with the protoxylem elements of the axis, although they resemble the latter in form, and in the appearance of the reticulum on their walls.

5. The nodal wood of the rhizome is poorly developed as compared with that of the aerial stem, and there are indications that it develops late in the ontogeny.

6. Interspersed among the nodal tracheids are parenchymatous cells of the same shape as the tracheids. These retain their cell contents.

7. In some of the nodal tracheids the lumen is nearly or completely obliterated by sclerized thickening of the cell wall.

8. In the neighborhood of the node, somewhat below the departure of the traces, the carinal canals may contain a substance of an apparently mucilaginous nature.

9. The leaf traces are very minute. Their xylem usually consists of 2–4 reticulate tracheids given off by the protoxylem. In the cortex the traces become concentric.

10. The root bearing branches of the rhizome may remain undeveloped. In the specimen in question there were no indications of

vascular structure above the first diaphragm of the branch. Each
branch gave rise to two roots, the steles of which were given off from
exactly opposite points. The roots and their steles appeared to be
of the same age.

11. All the internodal portions available for study were mature,
so that it is not possible to state whether the elements of the inter-
nodal lateral strands of metaxylem developed centripetally or
centrifugally.

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# POLLEN MORPHOLOGY AS AN INDEX TO PLANT RELATIONSHIP

## I. MORPHOLOGY OF POLLEN

### MAXY ALICE POPE

#### (WITH PLATE VIII)

### Introduction

In the summer of 1922 the writer began to devise a key whereby pollen of the common native flowers of northern Colorado could be identified microscopically. As the work progressed, it broadened out and much information accumulated, both as to native and introduced species. This material was arranged by families and formed an extended account, from which the present paper has been extracted. It is hoped that this larger systematic account may be published at some later time.

A grant from the National Research Council made it possible to undertake this work. My thanks are due to DR. FRANK E. LUTZ for suggesting the study, and also to Professor T. D. A. COCKERELL for material which he has furnished. To Professor FRANCIS RAMALEY I am under special obligations for his continued interest and valuable suggestions during the progress of the work.

Very little literature was found pertaining to the morphology of pollen. KERNER and OLIVER[1] give a brief account of the subject. A book by EDGEWORTH,[2] published in 1877, has about 500 illustrations, but unfortunately it is impossible to determine which figures belong to a particular description. ENGLER and PRANTL[3] figure pollen of many families of plants. The most recent publication dealing with the subject of pollen is a book by SCHEPPEGRELL,[4] which contains many microphotographs of the more common hayfever producing plants. Nearly all textbooks of botany have some figures and brief description of pollen.

[1] KERNER and OLIVER, The natural history of plants. Vol. II. New York. 1895.

[2] EDGEWORTH, M. P., Pollen. London. 1877.

[3] ENGLER, A., and PRANTL, K., Natürlichen Pflanzenfamilien. Berlin. 1897.

[4] SCHEPPEGRELL, WILLIAM, Hayfever and asthma. 1922.

## Methods

Many methods for the preservation of pollen grains were tried. Most of them proved unsatisfactory, presumably because of the differences in osmotic pressure of preservative and pollen grain. The following method was finally adopted. The pollen was shaken from the mature stamen upon a clean slide. A clean cover-glass was ringed with balsam, and the grains mounted dry. Balsam mounts also were usually made of each pollen. In no case was the shrinkage of any pollen noted either in the dry mounts or in balsam. Perhaps the most common artefact is the swelling of ellipsoidal, pyramidal, and polyhedral grains in balsam, producing in such cases a spherical shape. Hence for correct determinations the balsam mounts are practically worthless, unless the grain was originally of the spherical type. Balsam mounts do have a certain value, however, in that in them it is possible to distinguish any peculiar nuclear structures or differences in the relative thickness of the coats.

## Investigation

### Grains separate and in sacs

The great majority of flowering plants produce their pollen grains in large quantities, but each grain is separate from every other. Such is the condition in Poaceae, Ranunculaceae, Rosaceae, Fabaceae, Polemoniaceae, Scrophulariaceae, Compositae, and many other families. There are exceptions, however, some families producing grains in definite sacs, as the Orchidaceae and Asclepiadaceae, two widely separated families. One of the families studied, Onagraceae, has grains held together by viscid protoplasmic threads. All of its members thus far examined have this marked characteristic. These grains are well characterized by their shape; they are pyramidal, and the threads appear to be attached to each of the apices of the grain. In the Ericaceae the mature pollen grains occur not singly, but in tetrads. In the four families just mentioned there are relatively few grains produced per flower. The special devices which they possess are valuable, in that there is an increased assurance of the grains being carried from one plant to another by insect visitors, and since fewer grains come to nothing, fewer need be produced.

<div style="text-align:center">DRYNESS OR STICKINESS</div>

Pollen grains vary not only in shape, size, color, etc., but also in being either very dry and powdery or quite sticky in nature. This latter character seems to be correlated with the amount of nectar secreted by the plant. Those plants which produce large amounts of nectar have pollen grains which also seem to exude a sticky or sugary fluid. This is noticeable when one is making dry mounts of pollen, because the grains tend to remain in masses, held so by this sugary secretion. This condition, of course, should not be confused with that in the Orchidaceae, Onagraceae, or Asclepiadaceae. Some of the well known families producing sticky pollen grains are Iridaceae, Rosaceae, Fabaceae, and Compositae. Plants of these families for the most part are insect pollinated, so that the sticky pollen is an advantage.

Numerous families produce dry powdery pollen. In such cases the quantity greatly exceeds that in families in which the pollen is more or less sticky. Thus the Pinaceae, Poaceae, Salicaceae, Betulaceae, Zygophyllaceae, Chenopodiaceae, Ambrosiaceae, and others usually produce enormous quantities of dry powdery pollen. It is evidently advantageous for wind dispersal to have the grains light and dry.

<div style="text-align:center">SHAPE</div>

One of the best characters for use in a key seems to be that of shape. All of the pollens thus far examined fall into some half-dozen groups. The great majority of the families studied have pollen grains ellipsoidal or else cylindrical (capsule-shaped), as, for instance, Nymphaeaceae, Brassicaceae (fig. 13), Capparidaceae (fig. 8), Rosaceae, Fabaceae (fig. 15), Linaceae, Loasaceae, Ammiaceae, Gentianaceae, Hydrophyllaceae, Lamiaceae, Scrophulariaceae, Compositae, etc. The group of perhaps next consequence has spherical pollen grains, including Chenopodiaceae, Nyctaginaceae, Zygophyllaceae, Malvaceae (fig. 1), Papaveraceae, Campanulaceae, Ambrosiaceae (fig. 4), etc. In the third group are Poaceae with grains in the form of a truncated pyramid or frustrum of a pyramid (fig. 16), and Betulaceae, Alsinaceae, Grossulariaceae (fig. 18), Cactaceae, Cichoriaceae, etc., with polyhedric grains. The fourth group is very small, containing Cyperaceae, Juncaceae, Primulaceae (fig. 17),

Santalaceae, and Onagraceae (fig. 11), with pyramidal pollen grains. A final group includes a single family of those thus far studied, namely, Boraginaceae (fig. 14), with very small grains dumb-bell shaped.

The shape seems fairly constant for all of the pollens of any one family, excepting in a comparatively few cases. For example, the pollen grains of *Convolvulus americanus* and *C. interior* are spherical, whereas those of *C. arvensis* are ellipsoidal. All of the genera of Solanaceae thus far examined have ellipsoidal pollen grains, except *Salpiglossis*, which has grains made up of four united globes. In the Caprifoliaceae, *Lonicera* and *Linnaea* have spherical grains, while *Viburnum* has ellipsoidal grains; *Catalpa speciosa* has large spherical grains with numerous warty knobs, but *Tecoma* has ellipsoidal grains. In the Acanthaceae, according to the figures of ENGLER and PRANTL, we find spherical, ellipsoidal, oblong, and many different types and sizes of grain.

## SIZE

The size of pollen grains seems to be fairly constant in any species, and even among all of the species of a genus; often also among the genera examined in a family. This character is important in the identification of families by the key which has been worked out. In some cultivated plants there are often pollen grains of two distinct sizes, indicating perhaps a hybrid origin. This condition was noticed in the garden rose, tulip, and hothouse *Oxalis* for cultivated plants, and in *Douglasia montana* and *Sidalcea asprella* of the native plants.

The differences in size of pollen from various families is remarkable. Some grains are very large and visible to the naked eye, as is shown by the following measurements (in microns): *Zea mays* 160, *Humulus neomexicanus* 100–140, *Althaea rosea* 100, *Gossypium* 100, *Salpiglossis sinuata* 108. Other grains are so small as to require a compound microscope for detection, as in all of the Monotropaceae, Primulaceae, Apocynaceae, Santalaceae, and Boraginaceae. Examples are: *Hypopitys* 12, *Cyclamen europaeum* 16, *Apocynum* 22, *Mertensia lanceolata* 10.

## COLOR

The colors of pollen grains seem to be almost constant for any one family of plants, only one or two exceptions having been noted.

The colors vary from the deep orange-yellow characteristic of the members of the Portulacaceae, Fabaceae, Compositae, and others, to the pale, almost cream colors of such families as the Caryophyllaceae, Primulaceae, Polemoniaceae, and Bignoniaceae. Some grains are more or less bluish in color, as for example those of the Onagraceae, and some of the Polemoniaceae; others are characterized by a greenish color, noticeable in *Cleome* (Capparidaceae). The great majority of plant families seem to have pollen with a more or less uniform, ordinary yellow color, as Poaceae, Betulaceae, Ranunculaceae, Brassicaceae, Rosaceae, Malvaceae, Scrophulariaceae, etc.

Color alone, therefore, is insufficient for purposes of identification, although it helps if other characters are mentioned. The color probably is important in the insect world. It seems that those grains of a deep yellow, blue, or greenish color are produced on flowers which are chiefly insect pollinated, whereas some of the more common yellow ones are scattered by other means, principally the wind, as in Poaceae and Betulaceae.

### POLLEN WALL

As a rule, the wall of a pollen grain is three-layered. According to KERNER and OLIVER, these layers are the outer or perine, the middle or extine, and the internal or intine. The intine is usually very thin, and closely attached on one side to the extine and on the other to the protoplasm. The extine varies in thickness, apparently depending upon the shape of the pollen grain, but remaining constant for each particular shape. Thus all spherical grains have an extine which is usually more than $3 \mu$ thick, and in some cases $8-10 \mu$. Most of the grains having extines less than $3 \mu$ are either polyhedric or ellipsoidal in shape. Grains with thick extines are more apt to have pores than those with a thinner middle coat. The perine always is very thin, and is deposited from the matrix in which the young pollen grain is imbedded. It is closely associated with the extine, and is hard to distinguish. KERNER and OLIVER state that the various sculpturings, prickles, and other irregularities of the outer coat really appertain to the perine. In the present study the term extine includes both perine and extine.

## EXTERNAL MARKINGS

The best, most constant, and readily distinguishable character by which grains may be identified is smoothness or non-smoothness of the extine, or, in other words, the external marking of the grains. The divisions under which all grains seem to fall in regard to this character are extine smooth, reticulate, punctate, echinate, with pores.

EXTINE SMOOTH.—In many families the pollen grains have no characteristic markings, the surface appearing smooth, as in Poaceae (fig. 16), Commelinaceae, Betulaceae, Grossulariaceae (fig. 18), Rhamnaceae, etc. These families are further characterized, of course, by size, shape, and color.

EXTINE RETICULATE.—A great majority of grains have thickenings of the extine, which have a more or less definite arrangement or pattern. These thickenings are usually spoken of as reticulations. In some families the patterns or facets made by the reticulations are rather small and quite regular, as in Capparidaceae (fig. 8), Oxalidaceae, Zygophyllaceae, Linaceae, Caesalpinaceae, and Polemoniaceae; whereas in other families the reticulations are distinct, but more or less irregular, as in Nyctaginaceae (fig. 7), Saxifragaceae, Rosaceae, Vitaceae, Loasaceae, and Solanaceae. In some families reticulations are present together with pores, and it is not unusual to find a pore in the center of each facet.

EXTINE PUNCTATE.—The presence of pits or punctures on the pollen grain is very common, and occurs in Papaveraceae, Brassicaceae (fig. 13), Fabaceae (fig. 15), Scrophulariaceae, Lobeliaceae, etc.

EXTINE ECHINATE.—One of the most interesting of the variations manifested by pollen grains is found in the echinations or prolongations of the extine into definite spines. The number, length, and position of these spines vary in the different plant families in which they occur, and constitute some of the most exact characters for identification purposes. In some families the spines are very long and quite striking, as in all members of the Malvaceae (fig. 1) thus far examined and also in the Nymphaeaceae. All members thus far studied of Valerianaceae, Ambrosiaceae (fig. 4), and Compositae (fig. 6), however, have very much shorter and more numerous spines. Grains may also have pores along with these echinations, as in

Caprifoliaceae (*Lonicera* and *Linnaea*), Malvaceae, Campanulaceae, and Ambrosiaceae (these show only in balsam mounts).

The shape of the grain may be correlated with the presence of echinations, as all grains thus far found possessing spines are either spherical (Caprifoliaceae, Malvaceae, and Ambrosiaceae, figs. 1, 3, 4) or ellipsoidal (Nymphaeaceae, Valerianaceae, and Compositae, fig. 6). The dumb-bell, cubical, or polyhedric-shaped grains may have markings, but never prolongations of the extine into definite spines, except in the case of Cichoriaceae, in which the polyhedric grains do have spines (fig. 5).

EDGEWORTH noted echinate pollen grains in twenty-one families. The writer has found echinations in species from five of these families. These are placed first in his list which follows: Campanulaceae, Compositae, Valerianaceae, Caprifoliaceae, Nymphaeaceae, Smilaceae, Butomaceae, Taccaceae, Polygonaceae, Verbenaceae, Acanthaceae, Convolvulaceae, Ficoideae, Cactaceae, Droseraceae, Sapindaceae, Dicenaeaceae, Geraniaceae, Sterculiaceae, Berberideaceae, and Ranunculaceae. In addition to these, echinate grains have been observed in the present study in Ambrosiaceae and Malvaceae.

EXTINE WITH PORES.—Many of the families have no particular extension of the extine, but are decidedly different in that there are pores present. The size, number, and position of these pores vary with the different families. Families in which pores are conspicuous are Chenopodiaceae, Nyctaginaceae (fig. 7), Caryophyllaceae, Grossulariaceae (fig. 8), Malvaceae (fig. 1), Cactaceae, Onagraceae (fig. 11), and Campanulaceae. In some of these families the arrangement of the pores is striking, and in most cases constant. As an example may be named the Onagraceae, which so far as studied are found to have tetrahedral grains. There are four pores, one at each apex; usually only three of these are visible at one time (fig. 11). These are not complete openings in the extine, but simply thinner places in the outer covering. In Grossulariaceae (fig. 18) one large pore is present in the center of each one of the twelve faces of the grain. When the pollen tube develops it grows out through one of these pores.

### INTERNAL DIFFERENTIATION

Since the writer's chief interest has been in external morphology, very little time has been spent in a study of internal differentiation.

## TABLE I

CLASSIFICATION OF POLLEN GRAINS ACCORDING TO SHAPE, SIZE, AND EXTERNAL MARKINGS; S, SMALL GRAINS, (10–30 $\mu$); M, MEDIUM GRAINS (30–60 $\mu$); L, LARGE GRAINS (60–100 $\mu$)

| Kind of surface and markings | Elongated ellipse | More than one division | Spherical | Dumb-bell shaped | Ellipsoidal | Cylindrical (capsule-shaped) | Pyramidal | Polyhedric |
|---|---|---|---|---|---|---|---|---|
| Echinate | | | Malvaceae, L; Ambrosiaceae, S; Caprifoliaceae, M; Campanulaceae, S | | Nymphaeaceae, L; Valerianaceae, L; Dipsaceae, L; Compositae, M; Caprifoliaceae, M | | | Cichoriaceae, M |
| Reticulate | Convallariaceae, M; Melanthaceae, M; Amaryllidaceae, M; Alliaceae, M | Pinaceae, L | Polemoniaceae, M; Zygophyllaceae, M; Nyctaginaceae, M; Geraniaceae, L; Fumariaceae, S; Cannabinaceae, L; Bignoniaceae, L | | Cesalpiniaceae, M; Capparidaceae, S; Oxalidaceae, M; Oleaceae, M; Gentianaceae, M; Solanaceae, M; Menyanthaceae, L; Loasaceae, M; Saxifragaceae, S; Euphorbiaceae, M; Liliaceae, L; Iridaceae, L; Anacardiaceae, M; Cannabinaceae, L; Bignoniaceae, M | Rosaceae, M; Vitaceae, S | Juncaceae, M | |
| Smooth | Commelinaceae, M | Ericaceae, M; Apocynaceae, S | | Boraginaceae, S | Oleaceae, S; Araceae, M; Convolvulaceae, L; Rhamnaceae, M | Crassulaceae, S; Loganiaceae, S; Aizoaceae, M; Polygonaceae, M | | Betulaceae, S; Grossulariaceae, S; Monotropaceae, S; Poaceae, M |
| Punctate | Scrophulariaceae, M; Violaceae, L; Liliaceae, L | | Nyctaginaceae, L; Caryophyllaceae, M | | Dipsaceae, L; Amygdalaceae, S; Araliaceae, S; Scrophulariaceae, S; Brassicaceae, L; Lobeliaceae, M; Papaveraceae, M | Vitaceae, S; Fabaceae, S; Hydrophyllaceae, M | Onagraceae, L; Santalaceae, S; Primulaceae, S; Cyperaceae, S | Alsinaceae, M |
| Verruculose | Solanaceae, L | | Geraniaceae, L | | | | Onagraceae, L | Cactaceae, L; Cucurbitaceae, L; Ranunculaceae, S |

TABLE I—*Continued*

| Bands | | Acanthaceae, M | | Ammiaceae, M<br>Polygonaceae, M | Juncaceae, M | |
|---|---|---|---|---|---|---|
| Furrows or grooves | Scrophulariaceae, M<br>Violaceae, M<br>Liliaceae, L<br>Convallariaceae, M<br>Melanthaceae, M<br>Amaryllidaceae, M<br>Alliaceae, M | Fumariaceae, S | Oleaceae, M, S<br>Gentianaceae, M<br>Solanaceae, M<br>Menyanthaceae, L<br>Loasaceae, M<br>Saxifragaceae, S<br>Papaveraceae, M<br>Lamiaceae, S<br>Amygdalaceae, S<br>Araliaceae, S<br>Scrophulariaceae, S<br>Brassicaceae, M<br>Capparidaceae, S<br>Lobeliaceae, M<br>Oxalidaceae, M<br>Euphorbiaceae, M<br>Linaceae, L<br>Iridaceae, L<br>Anacardiaceae, L<br>Compositae, M<br>Convolvulaceae, L | Vitaceae, S<br>Fabaceae, S<br>Hydrophyllaceae, M<br>Crassulaceae, S<br>Loganiaceae, S | Santalaceae, S<br>Primulaceae, S | |
| With distinct pores | Malvaceae, L<br>Ambrosiaceae, S<br>Caprifoliaceae, M<br>Portulacaceae, M<br>Convolvulaceae, M<br>Papaveraceae, L<br>Geraniaceae, L<br>Caryophyllaceae, M<br>Amaranthaceae, S<br>Chenopodiaceae, S<br>Campanulaceae, S | | Valerianaceae, L | | Onagraceae, L | Grossulariaceae, S<br>Ranunculaceae, S<br>Alismataceae, M<br>Cucurbitaceae, L<br>Cactaceae, L |

A few facts have been noted, however, especially in regard to the nucleus, which seems in all cases to be central in position. The nucleus is quite easily seen in stained material, and if unstained material is properly examined can be distinguished with proper focusing. When unstained the nucleus appears darker and denser and in all ways like the nuclear structure of any typical plant cell. In regard to cytoplasmic structure, it seems, in the main, to resemble any young plant cell in which the entire space between nuclear membrane and cell wall is filled with a more or less homogeneous, alveolar material.

### TABULATION OF DIFFERENCES

Table I gives in condensed form much of the information obtained from examination of pollen grains of plants available for study. As will be noted, the same family may occur in more than one place in the table, because of differences in grains within the family.

### Summary

The paper reports a study of pollen morphology in numerous genera of about eighty families represented in the flora of Colorado, together with a small number of familiar exotic forms. A discussion is given of the general interest of pollen, with methods of study and preservation. Pollen shapes are described, as well as markings, size, color, stickiness, abundance, and other features.

It is found that, in general, the shapes and sizes of grains in the various genera of a family correspond rather closely, but there are some families which show striking differences. The most common shape of pollen grains among dicotyledons is short ellipsoidal. Most of the higher as well as many of the lower families have such ellipsoidal pollen. Other common shapes are spherical, pyramidal, polyhedral, etc. Among the monocotyledons, many families have grains elongated ellipsoidal. The same shape occurs also among some of the dicotyledons. In the Poaceae the grains are pyramidal or like a frustrum of a pyramid.

As to external features, it has been found possible to classify grains as echinate, reticulate, smooth, punctate, verruculose, etc. It is a conspicuous fact that many of the higher families of dicotyledons have echinate pollen.

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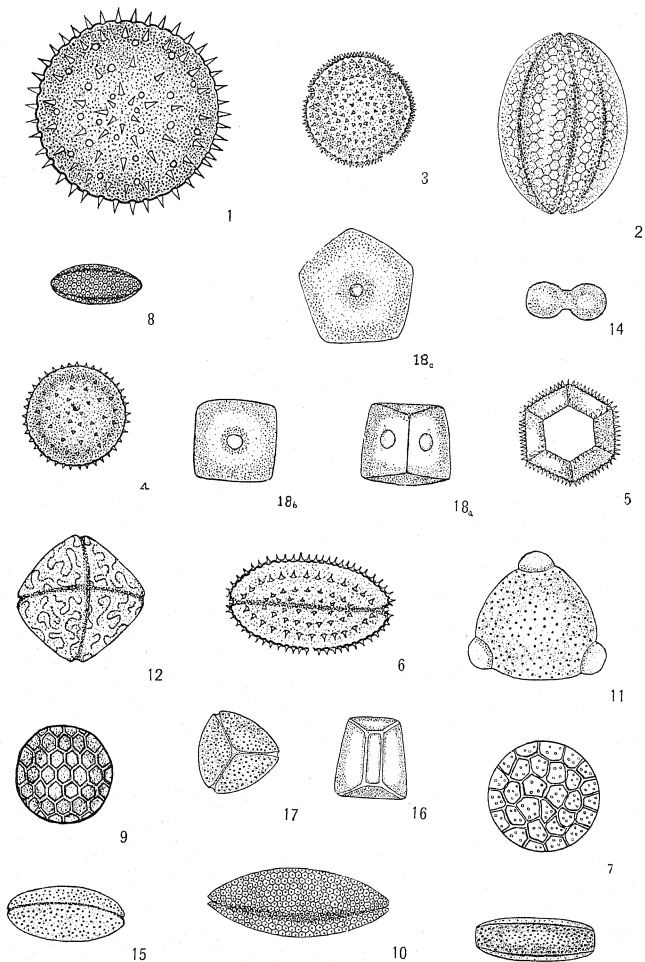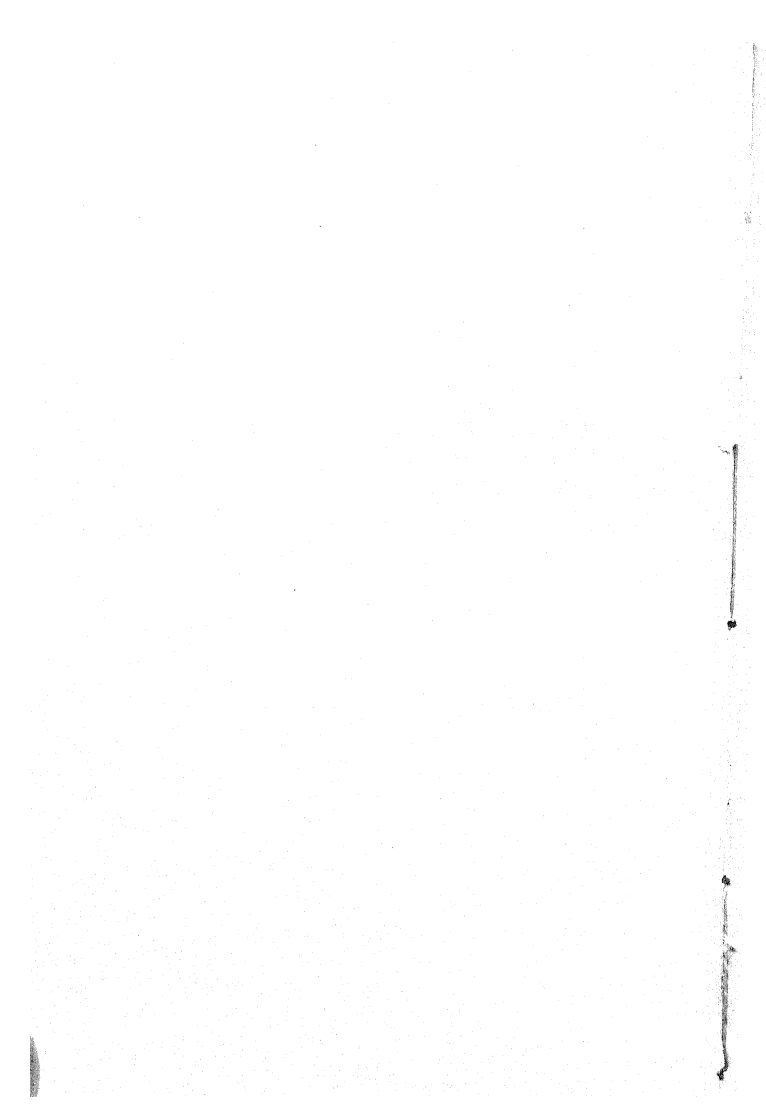
PLATE VIII

## EXPLANATION OF PLATE VIII

The figures were drawn with Abbe camera lucida, Bausch and Lomb 4 mm. objective and ocular 10. They were made from pollen grains mounted dry. The magnification of the figs. 1, 3, 5, 7, 9, 11, and 15 was 625; of figs. 2, 4, 6, 8, 10, 12, 13, 16, 17, and 18 *a, b, c,* 1250; of fig. 14, 1875. They are reduced approximately one-third in the process of reproduction.

FIG. 1.—*Sidalcea asprella* Greene (Malvaceae); large spherical echinate pollen grain; ×312.

FIG. 2.—*Monarda* sp.? (Lamiaceae); small ellipsoidal pollen grain with reticulations; ×625.

FIG. 3.—*Lonicera sempervirens* L. (Caprifoliaceae); medium sized spherical pollen grain with short echinations; ×312.

FIG. 4.—*Ambrosia elatior* L. (Ambrosiaceae); small spherical echinate pollen grain with pores; ×625.

FIG. 5.—*Nothocalais cuspidata* (Pursh) Greene (Cichoriaceae); medium sized polyhedral echinate pollen grain; ×312.

FIG. 6.—*Grindelia oregana* A. Gray (Compositae); medium sized or small ellipsoidal pollen grain with spines and pores; ×625.

FIG. 7.—*Abronia fragrans* Nutt. (Nyctaginaceae); medium sized pollen grain with reticulations; ×312.

FIG. 8.—*Peritoma angustum* (M. E. Jones) Rydb. (Capparidaceae); small ellipsoidal pollen grain with grooves and furrows; ×625.

FIG. 9.—*Tribulus terrestris* L. (Zygophyllaceae); medium sized spherical pollen grain with regular reticulations; ×312.

FIG. 10.—*Toxicoscordion falcatum* Rydb. (Melanthaceae); medium sized pollen grain, shape of an elongated ellipse, and having reticulated surface; ×625.

FIG. 11.—*Chamaenerion spicatum* (Lam.) S. F. Gray (Onagraceae); large tetrahedral pollen grain with large pores at corners; ×312.

FIG. 12.—*Capnoides montanum* (Engelm.) Britton (Fumariaceae); small spherical pollen grain with grooves; ×625.

FIG. 13.—*Draba streptocarpa* A. Gray (Brassicaceae); medium sized pollen grain with grooves; ×625.

FIG. 14.—*Mertensia brachyloba* Greene (Boraginaceae); small dumb-bell shaped pollen grain; ×937.

FIG. 15.—*Petalostemon purpureus* (Vent.) Rydb. (Fabaceae); small capsule-shaped pollen grain with punctate surface; ×312.

FIG. 16.—*Bromus Porteri* (Coult.) Nash (Poaceae); medium sized pollen grain shape of frustrum of pyramid; ×625.

FIG. 17.—*Primula Parryi* A. Gray (Primulaceae); small pyramidal pollen grain with punctate surface; ×625.

FIG. 18.—*Ribes longifolium* Nutt. (Grossulariaceae): *a,* side view; *b,* view of lateral face; *c,* face view of base; small polyhedric pollen grain with smooth surface and distinct pores; ×625.

# GROWTH OF WHEAT ROOTS IN SALT SOLUTIONS CONTAINING ESSENTIAL IONS[1]

Sam F. Trelease and Helen M. Trelease

(WITH TWO FIGURES)

Retardation of root growth has been used in many studies as an index of the injurious or toxic action of various chemical agents upon plant protoplasm. Roots are specially suitable for such studies, because their protoplasm is readily accessible to the toxic agent. The growth responses of roots immersed in solutions containing the ions that are essential for plant growth have received relatively little attention. Since the roots are the organs through which all of the salts usually enter the plant, the effects of nutrient salts upon root growth are of special physiological importance. These considerations have led the writers to an attempt to secure some quantitative data on the rate of elongation of wheat roots in aqueous solutions containing nutrient salts. The results indicate some of the relations that exist between the composition of the culture solutions and the growth made by the roots, and furnish information concerning the salt proportions that are favorable for root development.

## Method

The thirty-seven culture solutions used contained the three salts, potassium dihydrogen phosphate ($KH_2PO_4$), calcium nitrate ($Ca(NO_3)_2$), and magnesium sulphate ($MgSO_4$), in various sets of proportions. Each solution had a total volume-molecular concentration of 0.06 gram-molecule per liter, but the solutions differed in the volume-molecular proportions of the component salts. The volume-molecular proportions of the salts are given in table I, and the compositions of the solutions are represented graphically by means of points shown on the equilateral triangle of fig. 1. Near the points are numbers corresponding to the solution numbers in table I. The upper apex of the diagram represents a solution in which 100

[1] Botanical contribution from the Johns Hopkins University.

per cent of the dissolved molecules are $KH_2PO_4$; the left apex, one in which 100 per cent of these are $MgSO_4$; and the right apex, one in which 100 per cent of the solute molecules are $Ca(NO_3)_2$. Points on the base of the triangle denote various molecular proportions of $Ca(NO_3)_2$ and $MgSO_4$, the percentage of $KH_2PO_4$ being zero. Points on the left side represent solutions containing various molecular proportions of $KH_2PO_4$ and $MgSO_4$, these solutions containing no $Ca(NO_3)_2$. Points on the right side denote solutions containing $Ca(NO_3)_2$ and $KH_2PO_4$, but no $MgSO_4$. Points in the interior of the triangle represent solutions that contain all three salts.

Spring wheat of a pure line was employed in these tests (Marquis, Saskatchewan, no. 70, Selection no. 313, supplied by the University of Saskatchewan, through the kindness of Professor MANLEY CHAMPLIN). After soaking for three hours in tap water, the seeds were sprouted on wet blotting paper in a moist chamber. When the first root of each seedling was about 4 mm. long, the seedlings were transferred to the culture vessels, which were ordinary glass tumblers with a capacity of about 275 cc. A piece of paraffined bobbinet (somewhat like mosquito netting, but with hexagonal meshes and firmly fixed threads) was stretched over the top of the tumbler and fastened by a ligature of paraffined linen thread. The tumbler stood in a 600 cc. beaker (Griffin low form), and both the tumbler and the space around it were filled with the solution, the level of the latter being even with the top of the tumbler in both cases.

Fifty seedlings were used in each culture. They were placed upon the netting at the surface of the solution, care being taken that every root dipped into the solution and that the seeds were not flooded. The cultures were kept in darkness, and a moist chamber was provided for each during the first two days by covering the beaker with an inverted watch glass (10.5 cm. in diameter). When the roots in solution 23 (which has equal molecular proportions of the three salts) had attained a length of about 70 mm., all the plant-lets of cultures in the series were harvested, and the length of the longest root on each seedling was recorded.

These experiments were begun in May 1923, at the Laboratory of Plant Physiology of the Johns Hopkins University, and they were continued at the University of Louisville. In the different series the

temperature ranged from 18° to 23° C., and the corresponding time required for the roots in the standard solution to attain a length of about 70 mm. ranged from 89 hours to 69 hours. The writers are pleased to acknowledge indebtedness to Professor B. E. LIVINGSTON for suggestions in the preparation of this paper.
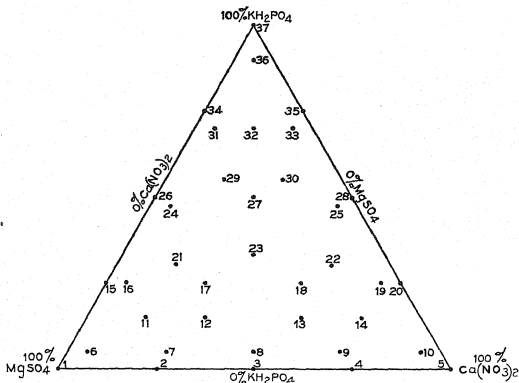


FIG. 1.—Triangular diagram showing culture numbers and volume-molecular salt proportions of one-, two-, and three-salt solutions.

## Results

The results of several series are summarized in table I. In each series from twelve to sixteen cultures usually were employed, and the relative amounts of growth made in the various solutions always were expressed as percentages of the corresponding growth in solution 23. The value given for each individual test (fifth column of the table) is based upon a mean for fifty seedlings. The average for each solution is the mean of all individual values for that solution. The relative physiological values of the various solutions were derived by expressing each value in the preceding column as a percentage of the highest value in that column.

To bring out relations between root growth and the molecular

proportions of the salts employed, relative physiological values are shown on the triangular diagram of fig. 2, which is similar to fig. 1 except that the physiological values replace the culture numbers. The contour lines on the diagram have been drawn so as to divide the area of the triangle into seven zones, representing the following
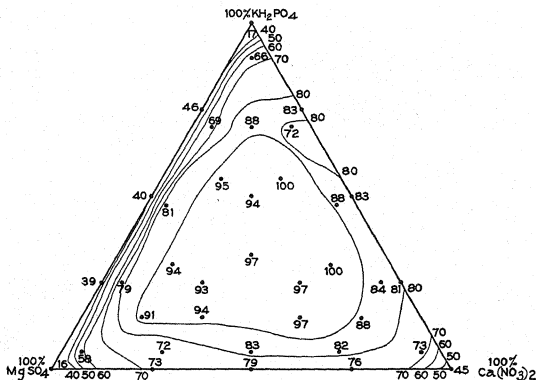


FIG. 2.—Relative physiological values of solutions; contour lines divide triangular area into zones of physiological values as related to salt proportions.

ranges of physiological values: 90–100, 80–90, 70–80, 60–70, 50–60, 40–50, and below 40.

The zoning brought out by these contours is very regular and consistent, the only noticeable break in the regular concentricity of the zones being that necessitated by the value shown for solution 33. The eccentricity suggested at that point on the diagram may well be significant, or it may be related only to the group of unknown experimental features connoted by the term experimental error. At any rate, the data offer no means for detailed study of the slight eccentricity in question. Considering the limitations of such experimentation as this,'the zoning appears to be as consistent as might be hoped for.

The central zone indicates high relative physiological values (from 90 to 100) for a group of eleven solutions, nos. 11, 12, 13, 17,

TABLE I

SALT PROPORTIONS OF CULTURE SOLUTIONS AND CORRESPONDING RELATIVE AMOUNTS OF GROWTH MADE BY ROOTS OF WHEAT SEEDLINGS

| SOLUTION NO. | RELATIVE VOLUME-MOLECULAR PROPORTIONS OF SALTS | | | LENGTH OF LONGEST ROOT AS PERCENTAGE OF LENGTH FOR CONTROL CULTURE IN EACH CASE* | | RELATIVE PHYSIOLOGICAL VALUE OF SOLUTION |
|---|---|---|---|---|---|---|
| | $KH_2PO_4$ | $Ca(NO_3)_2$ | $MgSO_4$ | Individual tests | Average | |
| 1 | 0.0 | 0.0 | 100.0 | 15, 17 | 16 | 16 |
| 2 | 0.0 | 25.0 | 75.0 | 71, 79 | 75 | 73 |
| 3 | 0.0 | 50.0 | 50.0 | 78, 83 | 81 | 79 |
| 4 | 0.0 | 75.0 | 25.0 | 75, 81 | 78 | 76 |
| 5 | 0.0 | 100.0 | 00.0 | 44, 47 | 46 | 45 |
| 6 | 5.0 | 5.0 | 90.0 | 55, 60, 59, 58, 59, 65, 63 | 60 | 58 |
| 7 | 5.0 | 25.0 | 70.0 | 77, 84, 68 | 74 | 72 |
| 8 | 5.0 | 47.5 | 47.5 | 80, 89, 85 | 85 | 83 |
| 9 | 5.0 | 70.0 | 25.0 | 81, 82, 90 | 84 | 82 |
| 10 | 5.0 | 90.0 | 5.0 | 65, 72, 75, 78, 78, 83 | 75 | 73 |
| 11 | 15.0 | 15.0 | 70.0 | 94, 93 | 94 | 91 |
| 12 | 15.0 | 30.0 | 55.0 | 95, 98 | 97 | 94 |
| 13 | 15.0 | 55.0 | 30.0 | 100, 100 | 100 | 97 |
| 14 | 15.0 | 70.0 | 15.0 | 89, 93 | 91 | 88 |
| 15 | 25.0 | 0.0 | 75.0 | 38, 41 | 40 | 39 |
| 16 | 25.0 | 5.0 | 70.0 | 76, 89, 78 | 81 | 79 |
| 17 | 25.0 | 25.0 | 50.0 | 90, 97, 94, 102, 99 | 96 | 93 |
| 18 | 25.0 | 50.0 | 25.0 | 95, 103, 99, 101, 103 | 100 | 97 |
| 19 | 25.0 | 70.0 | 5.0 | 86, 85, 86 | 86 | 84 |
| 20 | 25.0 | 75.0 | 0.0 | 82, 83 | 83 | 81 |
| 21 | 30.0 | 15.0 | 55.0 | 96, 98 | 97 | 94 |
| 22 | 30.0 | 55.0 | 15.0 | 101, 104 | 103 | 100 |
| 23 | 33.3 | 33.3 | 33.3 | 100 (control)† | 100 | 97 |
| 24 | 47.5 | 5.0 | 47.5 | 84, 81, 83 | 83 | 81 |
| 25 | 47.5 | 47.5 | 5.0 | 90, 92, 90 | 91 | 88 |
| 26 | 50.0 | 0.0 | 50.0 | 38, 43 | 41 | 40 |
| 27 | 50.0 | 25.0 | 25.0 | 93, 98, 93, 99, 103 | 97 | 94 |
| 28 | 50.0 | 50.0 | 0.0 | 82, 87 | 85 | 83 |
| 29 | 55.0 | 15.0 | 30.0 | 96, 99 | 98 | 95 |
| 30 | 55.0 | 30.0 | 15.0 | 97, 108 | 103 | 100 |
| 31 | 70.0 | 5.0 | 25.0 | 69, 72, 72 | 71 | 69 |
| 32 | 70.0 | 15.0 | 15.0 | 92, 89 | 91 | 88 |
| 33 | 70.0 | 25.0 | 5.0 | 82, 72, 68 | 74 | 72 |
| 34 | 75.0 | 0.0 | 25.0 | 43, 51 | 47 | 46 |
| 35 | 75.0 | 25.0 | 0.0 | 83, 86 | 85 | 83 |
| 36 | 90.0 | 5.0 | 5.0 | 65, 58, 72, 74, 73, 67 | 68 | 66 |
| 37 | 100.0 | 0.0 | 0.0 | 19, 17 | 18 | 17 |

* Duration of each experiment was the time period (approximately 88 hours at 18.5° C.) required for roots in the control solution (no. 23) to increase in length from 4 mm. to 70 mm.

† Length of longest root in control culture was taken as 100 in each case.

18, 21, 22, 23, 27, 29, and 30. Any point within this zone may be expected to represent a three-salt solution with a physiological

value of 90 or above (on the criteria here used). Furthermore, solutions 14, 25, and 32 gave physiological values of 88, only slightly lower than the lowest ones included in the central zone on the diagram, and the points for these lie close to the central zone. These three solutions may be added to the eleven preceding, and thus secure an optimum group of fourteen solutions, every one of which gave a physiological value of 88 or above.

The central area defined by these fourteen points may be examined with reference to the sets of salt proportions that are included. These solutions generally are characterized by having the partial molecular concentration of every salt as great as or greater than 15 per cent of the total molecular concentration. There is but one exception, solution 25, in which the partial concentration of magnesium sulphate is only 5 per cent of the total concentration. On account of this exception, it is probably unsafe to predict that physiological values lower than 88 may be expected for all solutions in which the partial molecular concentration of any salt is less than 15 per cent of the total molecular concentration. The generalization seems to be well indicated, however, that all three-salt solutions of this type and total molecular concentration may be expected to have physiological values of 88 or above (with the non-solution conditions and by the criteria here used), provided the partial molecular concentration of no salt is lower than 15 per cent of the total molecular concentration.

It is of interest to consider whether the data for the optimum group of solutions are adequate for an attempt at further subdivision of this group. The central area of the diagram might be subdivided by additional contour lines representing values between 90 and 100, but such subdivisions surely would be transgressing the logical limits set by the degree of precision attained in the tests themselves. The data suggest that the very best solutions may be represented by points lying to the right of the vertical diameter of the triangle, and there seems to be indication that the solution values become progressively lower toward the left corner of the optimum area. The central area must represent the surface of a plateau with definite curvatures. It is apparent from the data that no three-salt solution of this type, with a total concentration of 0.06 gram-molecule per

liter, if tested with these seeds and these non-solution conditions, would be apt to give markedly more rapid root elongation than actually was observed for solutions 22 and 30. The generalization, however, seems to be about as precise as should be attempted at present.

The molecular ratios were calculated for the eleven tested solutions of the central area. The values of the ratio of calcium nitrate to magnesium sulphate range from 0.21 to 3.67, those of the ratio of potassium dihydrogen phosphate to magnesium sulphate have the same range, and those of the ratio of calcium nitrate to potassium dihydrogen phosphate lie between 0.27 and 3.67. These results lead to the same conclusion as has been reached from experiments with longer culture periods and for later developmental phases, by GILE (1), TOTTINGHAM (3), SHIVE (2), TRELEASE (4), and others. The specific effect of any molecular ratio between two salts in a culture solution surely depends upon the complex balance of the partial concentrations of all three salts and their resulting ions. The influence of any pair of salts must be considered as determined by the relation of these salt molecules to those of the third salt, and doubtless to all other influential conditions. Theoretically, it should be possible to state an optimum range for the ratio between any two salts or between any two ions when the rest of the environmental complex is sufficiently restricted. If the rest of the complex is sufficiently altered, however, a corresponding change in the optimum range is to be expected.

If the solutions represented by the right-hand half of the optimum area really are to be considered as better than those represented by the left-hand half, then it is suggested that this feature is related to the ratio of calcium nitrate to magnesium sulphate, within the limits of the optimum area. The data do not warrant any discussion of this suggestion, however.

The relative physiological values (16 and 17) given for the simple solutions of magnesium sulphate and potassium dihydrogen phosphate (1 and 37) were the lowest of the series, but the solution (5) containing only calcium nitrate has a much higher value (45). These three determinations, which are represented by the three apices of the triangle in fig. 2, constitute a simple study of the toxic-

ity of the three salts in question, when employed with a concentration of 0.06 gram-molecule per liter. It appears that, for initial root growth under the conditions of these tests, the first two salts were about twice as effective in retarding growth as was the third.

The series includes tests of each of the pairs of salts, the nine two-salt solutions represented on the margins of the diagram of fig. 2. The results of these tests furnish evidence of what has been called antagonism, but no analyses of this can be made from the data of only three combinations of each pair of salts. It is interesting to note that the physiological values of the three combinations of calcium nitrate and potassium dihydrogen phosphate, and the three of calcium nitrate and magnesium sulphate are all about the same (from 73 to 83), while the values for the three combinations of magnesium sulphate and potassium dihydrogen phosphate are much lower (39 to 46). These general relations might be expected from the relative toxicities of the single-salt solutions. Combinations of the two very toxic salts give much greater growth retardation than do those of either one of the very toxic salts with the slightly toxic one.

One of the conspicuous features of the zonation shown in fig. 2 is the excessive crowding of the contours near the left margin, which represents a marked fall in physiological values in passing from solutions with 5 per cent of their total molecular concentration due to calcium nitrate to those that contain none of that salt.

In the present paper no discussion is attempted of zonation, etc., with reference to the many theories of physiological and physical chemistry that might be applied to these results. These questions may be left to some future time, after there is sufficient evidence that the results themselves are reliable enough to warrant such discussion.

It should be mentioned that with other experimental technique, other solution volumes or rates of renewal, other total concentrations, other phases of development, other kinds of plants, other temperatures, etc., the comparative values obtained might have been very different from those secured in these studies. TRELEASE and LIVINGSTON (5) have emphasized the important influence that climatic conditions may exert in experiments with solution cultures.

In conclusion, attention may be called especially to the high degree of consistency shown by these results. Many of the inconsistencies so frequently encountered in the study of experimental results with solution cultures unquestionably are due to the extreme complexity of both the internal and the external complexes of influential conditions. A study of the initial behavior of germinating seeds may be expected to involve less complexity, within as well as outside the organism, than would be involved in a study of later phases of growth. Notably, the experiment period could be very short in the experiments here described, thus practically avoiding many of the alterations in organisms and solution that increase with time. Also, the whole question of photic environment was avoided, since such tests can be carried on in darkness. It appears that this general type of experimentation is very promising, and that really reliable solution-culture results (in the sense of physics and chemistry) may be secured if attention is confined to the first few days of seed germination. Our greatest present need in biological experimentation is that plans be worked out which will allow problems to be attacked in their very simplest form. Perhaps the main use of this paper may be that it contributes a little in that direction.

## Summary

1. This paper reports a study of root growth in very young wheat seedlings supplied with solutions containing one or more of the salts potassium dihydrogen phosphate, calcium nitrate, and magnesium sulphate.

2. Thirty-seven different solutions were tested, each with a total concentration of 0.06 gram-molecule per liter. Besides the 3 single-salt solutions, the series included 9 two-salt solutions and 25 three-salt solutions.

3. Marked retardation of root elongation did not occur unless the volume-molecular concentration of at least one of the three salts constituted less than about 15 per cent of the total volume-molecular concentration of the solution.

4. The roots were not very sensitive to small differences in salt proportions, except when the partial concentration of calcium nitrate in the solution was below about 5 per cent of the total concentration.

5. The results are presented by means of triangular co-ordinates, and they are very consistent.

6. It appears that root elongation in very young seedlings furnishes a subject that is not too complex for experimentation aiming toward reliable results.

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# COTYLEDON FORM AND SIZE IN RECIPROCAL HYBRIDS BETWEEN SPECIES OF DIGITALIS[1]

## J. BEN HILL

### (WITH FOUR FIGURES)

Seedlings of the species of *Digitalis* are characterized by two general types of cotyledons. The type characteristic of *D. purpurea* and *D. Thapsi* is small and short, distinctly broader than long; the other type of cotyledon, characteristic of *D. ambigua*, *D. lutea*, and others, is larger and in general slightly longer than broad.

During several years' study of species hybrids of *Digitalis*,[2] in crosses between various species having these distinct types of cotyledons, I have noted that the reciprocal $F_1$ hybrids differ considerably in the form and size of their cotyledons, and that they always resemble the female or seed parent in these respects. In fact, a resemblance to the seed parent, although difficult to describe, is apparent even in hybrids between species having the same type of cotyledon. In hybrids between those species differing in cotyledon form and size the difference is striking. The only reference found in the literature concerning a matroclinous tendency in the cotyledon characters of hybrids is that of FOCKE,[3] in his discussion of *Nymphaea* species hybrids, which he says have cotyledons resembling the female parent.

### Investigation

Reciprocal hybrids between *Digitalis purpurea* and *D. lutea* were secured in abundance in 1923. These are distinct species. differ-

ing markedly in the form and size of the cotyledons, and their hy-
brids furnished excellent material for the study. The customary
technical precautions were observed in making the hybridizations
and in growing the seedlings. Seedlings of the reciprocal $F_1$ hybrids
and of both parents were grown. Full maturity of the cotyledons of
*Digitalis* seedlings is reached within three or four weeks from plant-
ing. The observations were made at intervals of about a week, and
a series of stages in the development of the cotyledons was secured
and studied. Notes concerning the general appearance of the seed-
lings were made, and sketches to illustrate their resemblances and
differences. The drawings, of which the accompanying figures show
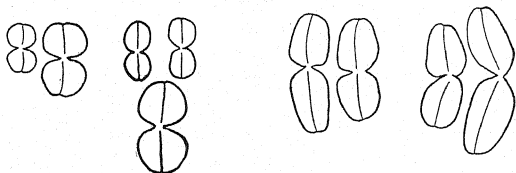


FIG. 1.—Very young stages of cotyledons about one week from date of planting;
parent species at either side and reciprocal hybrids between; reading from left to right:
*D. purpurea, D. purpurea*×*D. lutea, D. lutea*×*D. purpurea*, and *D. lutea*.

appropriate reductions, were camera lucida sketches showing a uni-
form magnification of ten diameters.

The cotyledons of *D. purpurea* are orbicular in the young stages,
with the transverse and longitudinal axes about equal (fig. 1). As the
cotyledons reach maturity, the transverse axis becomes greater than
the longitudinal axis (figs. 3, 4). Based on an average of twenty
measurements, the ratio of width to length was found to be 1.26 mm.
to 1.21 mm. for the young stages. Based on an average of thirty
measurements of mature cotyledons, the ratio of width to length was
found to be 5.25 mm. to 4.016 mm. These ratios, expressed in per-
centages, are 1.045 and 1.306 respectively. The tips of the cotyle-
dons are often retuse. The cotyledons of *D. lutea* are elliptic in the
young stages (fig. 1), and become broadly ovate in the older stages
(figs. 3, 4). The transverse axis is shorter than the longitudinal axis
in all stages. The ratio of width to length in the young stages, based

on an average of twenty measurements of young cotyledons, is
1.84 mm. to 2.55 mm. The ratio of width to length in the older
stages is 4.63 mm. to 4.85 mm., based on twenty measurements of
mature cotyledons. These ratios, expressed in percentages, are 0.722
and 0.954 respectively. The tips of the cotyledons are retuse to
emarginate, with a peculiar narrowing of the blade just behind the
tip.

Under favorable conditions, seeds of *Digitalis* germinate in from
five to eight days, seeds of pure species germinating more quickly
than seeds resulting from a species cross. The cotyledons reach
maturity in from three to four weeks from the date of planting. The
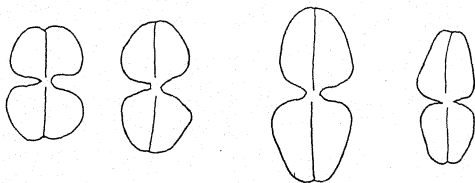


FIG. 2.—Young cotyledons about two weeks from date of planting, arrangement as
in fig. 1; the *D. lutea*×*D. purpurea* $F_1$ hybrid has cotyledons decidedly larger than its
reciprocal.

$F_1$ seedlings of *D. purpurea*×*D. lutea*, and those of the reciprocal
combination *D. lutea*×*D. purpurea*, were carefully compared with
each other, and with the parents in the very early stages, and these
observations were continued at intervals of about one week from the
date of planting to full maturity.

The cotyledons of *D. purpurea*×*D. lutea* are orbicular-elliptic in
shape, greatly resembling those of *D. purpurea*, but showing the
influence of the *D. lutea* parent to a slight extent. The ratio of width
to length in the young stages of the cotyledons of these $F_1$ hybrids,
based upon an average of twelve measurements, is 2.46 mm. to
2.58 mm. The ratio expressed in percentage is 0.961. The slight
influence of the *D. lutea* parent is expressed in the slight elongation
of the cotyledon. The seedlings of *D. purpurea*, which in this in-
stance is the female parent, have cotyledons, which even in the

young stages are slightly broader than long, the ratio being 1.26 mm. to 1.21 mm., or 1.045 per cent. The resemblance in both shape and size of these $F_1$ hybrids to the female parent is maintained to full maturity, but decreases with the full development. At maturity in the $F_1$ hybrids the ratio of width to length, based upon the average of twenty measurements of mature cotyledons, is 4.413 to 4.3125. This ratio expressed in percentage is 1.023 (table I). This ratio, as well as the shape (figs. 3, 4), indicates that in this hybrid combination the
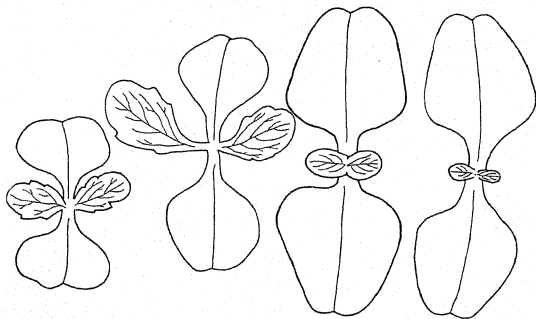


FIG. 3.—Mature cotyledons about three weeks from date of planting, arrangement as in fig. 1; true leaves beginning to appear; difference in size conspicuous in cotyledons at this stage.

matroclinous tendency as regards the form of the cotyledon is greater in the younger stages, for the cotyledons of the female parent are much broader than long. As the seedlings develop, the cotyledons remain smaller in this combination than in the reciprocal, and in general are about the size of the *D. purpurea* parent and much smaller than the *D. lutea* parent. As will be mentioned later, the feature of size is in sharp contrast with the reciprocal hybrid. At the end of two weeks the cotyledons of the $F_1$ hybrids of *D. purpurea*×*D. lutea* are scarcely more than one-half the size of the reciprocal. This difference in size occurred in several sets of sibs, the result of different hybridizations, and in one instance the $F_1$ hybrid seedlings were

even smaller than the *D. purpurea* seed parent. In general, the seed-
lings of this cross were about the same size as the seed parent in the
earlier stages. They become slightly larger than the seed parent
as they reach maturity.

Throughout their development from the earliest stages to full
maturity, the cotyledons of the F₁ hybrid of *D. lutea* × *D. purpurea*,
the reciprocal of the preceding combination, greatly resemble the *D.
lutea*, which in this case is the female or seed parent. In both form
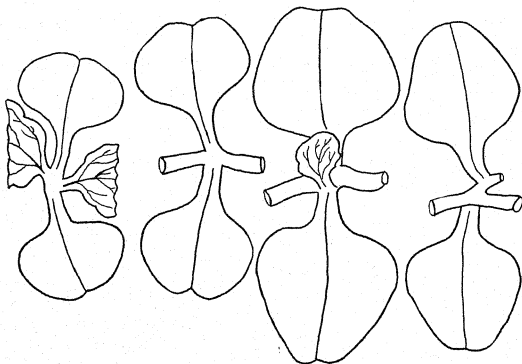


FIG. 4.—Fully mature cotyledons about four weeks from date of planting; arrange-
ment as in fig. 1; petioles of true leaves shown; differences in size of cotyledons in recip-
rocals maintained to full maturity.

and size the resemblance of the cotyledons of these hybrids to the
female parent is striking. In shape the cotyledons of the F₁ seedlings
of this combination are elliptic in the younger stages, and become
ovate to broadly ovate at maturity. In the younger stages, one week
to nine days old, the cotyledons are elongated and appear to be
almost identical with the *D. lutea* parent (figs. 1, 2). The ratio of
width to length in young stages is 3.88 mm. to 3.84 mm., or 0.88 per
cent, based upon an average of ten measurements. This compares
with a ratio of 1.84 mm. to 2.55 mm., or 0.722 per cent for the *D. lutea*

parent. A slight influence in the $F_1$ hybrid of the *D. purpurea* parent, in which the cotyledons are broader than long, is expressed in a comparative shortening of the cotyledons. As full maturity is reached, the cotyledons become more nearly like the *D. lutea* parent (figs. 3, 4). The ratio of width to length, based upon the average of seven measurements of mature cotyledons, is 5.58 mm. to 5.67. Expressed in percentage, this ratio is 0.984. This compares with a ratio of 4.63 mm. to 4.85 mm., or 0.954 per cent for the *D. lutea* parent (table I). The figures and table I, showing the ratios of width to length, furnish convincing evidence of the striking resemblance of this hybrid combination to the maternal type as regards cotyledon form.

In size, the seedlings of $F_1$ hybrids of *D. lutea*×*D. purpurea* are larger and more vigorous than the reciprocal combination, and at maturity they are larger than the *D. lutea* parent. This disparity in size in the reciprocals is noticeable in all stages of development, and especially so after about two weeks from planting. It is interesting to note that the matroclinous tendencies so strong in the cotyledons are not extended to the true leaves of the seedling. The third, fourth, and fifth leaves were observed in the seedlings of reciprocal hybrids of *D. purpurea* and *D. lutea*, and in all cases the leaves of the seedlings in reciprocals were identical.

TABLE I

COTYLEDON WIDTH AND LENGTH IN PARENT SPECIES AND RECIPROCAL $F_1$ HYBRIDS

| STAGE | D. PURPUREA | | | D. PURPUREA×D. LUTEA F₁ HYBRID | | | D. LUTEA×D. PURPUREA F₁ HYBRID | | | D. LUTEA | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Width in mm. | Length in mm. | Ratio width to length in percentage | Width in mm. | Length in mm. | Ratio width to length in percentage | Width in mm. | Length in mm. | Ratio width to length in percentage | Width in mm. | Length in mm. | Ratio width to length in percentage |
| Young | 1.26 | 1.21 | 1.045 | 2.46 | 2.55 | 0.961 | 3.38 | 3.84 | 0.880 | 1.84 | 2.55 | 0.722 |
| Mature | 5.25 | 4.016 | 1.306 | 4.443 | 4.3125 | 1.023 | 5.58 | 5.67 | 0.984 | 4.6275 | 4.85 | 0.954 |

I have grown reciprocal hybrids of this combination to maturity and found the mature reciprocal hybrids to be identical. The *D. lutea* type seems to be largely dominant in shape of leaf, but the plants are intermediate in many respects. The matroclinous tendencies, therefore, stop abruptly with the maturity of the cotyledons. The peculiarity of the inheritance of cotyledon size and form is the only instance of matrocliny observed in *Digitalis* species hybrids, and I do not regard matroclinous tendencies as characteristic of $F_1$ hybrids of species of this genus.

It would be interesting to follow the inheritance of the cotyledon form and size through the $F_2$ and later generations, but due to the extreme sterility of *Digitalis* $F_1$ interspecific hybrids, the hybrids of this genus do not furnish suitable material for the study of cotyledons in later generations. In all the mass of plant genetical material now under investigation in the various laboratories, there should be some species, varieties, or strains showing differing cotyledon characters and yielding fertile $F_1$ hybrids. Such forms might furnish suitable material for the further study of the inheritance of cotyledon characters.[4] Doubtless data are already in the hands of investigators concerning cotyledon characters.

### Discussion

Naturally the question of the cause of the matroclinous tendency of the cotyledon form and size in these $F_1$ hybrids arises. The situation is not parallel to those instances in animal forms, in which the hybrids of wide crosses show matroclinous tendencies. In the sea urchins of the echinoderms, where wide crosses are made, embryos of purely maternal characteristics result. The cause of this condition is the entire failure of the sperm to function in any capacity save as that of an activating agent. NEWMAN[5] states that this matroclinous situation is responsible for the traditional belief in the parthogenetic nature of heterogenetic hybrids, and has shown that it does not ob-

[4] Since the preparation of this manuscript, through the cooperation of Dr. SINNOTT and Mr. DURHAM, I have secured hybrids of homozygous strains of squash differing in cotyledon size, of which a study is being made.

[5] NEWMAN, H. H., Hybrids between *Fundulus* and *Mackerel*. Jour. Exp. Zool. **26**: 391–422 1918.

tain in that group of fishes known as the teleosts, in which the $F_1$s are true hybrids. The *Digitalis* hybrids under consideration are true hybrids, as shown by their growth to full maturity upon two different occasions.

In other instances, certain maternal characteristics in the early stages of the embryo of wide crosses in animal forms are accounted for on the basis of failure of the paternal chromatin to be assimilated in time to influence the developing embryo. This explanation has been made especially for the rate of growth of the embryo, or the "cleavage tempo."[6] NEWMAN's work, however, would seem to raise doubts as to the universal application of this principle. In any event, this explanation could scarcely be applied to the characteristics of mature cotyledons of plant embryos, for these are very late embryonic stages. Further, the third, fourth, fifth, and later leaves show perfect intermediate characteristics. Even the cotyledons, as is indicated, show to some extent the influence of the paternal parent in form.

It has been suggested to me by Dr. G. H. SHULL that the cause lies in the particular manner in which the seed coats, by compressing the cotyledons, determine their shape. The seed coats being formed from the integuments of the ovule, are entirely maternal in structure, and wholly uninfluenced by the hybridization which forms the embryo. The size of the seed and possibly the size of the embryo are apparently not unrelated to the size of the ovule. The size of the seed of *D. purpurea* differs from the size of that of *D. lutea*, the latter being three or four times the size of that of *D. purpurea*. The mature seed of *Digitalis* is well supplied with endosperm, however, which surrounds the embryo. In the developing stages the endosperm is soft tissue, and there may be objections to the idea of its compressing the embryo sufficiently to affect permanently the shape of the cotyledons of the embryo, even though the hard seed coats were restricting the whole developing structure.

Possibly the exact cause of the matroclinous tendencies of the cotyledons of these hybrids awaits further studies of the genetics of juvenile stages of plants, upon which there are as yet few data.

[6] MORGAN, T. H., Heredity of embryonic characters. Sci. Mo. 18:5–17. 1924.

## Summary

1. There are two distinct types of cotyledons in the seedlings of the various species of *Digitalis*. *D. purpurea* and *D. Thapsi* are characterized by cotyledons orbicular in shape, generally broader than long. *D. ambigua* and *D. lutea* and others are characterized by cotyledons elliptic to ovate, becoming broadly ovate at maturity, generally longer than broad.

2. $F_1$ hybrids between species with these different types of cotyledons show strong matroclinous tendencies. Matrocliny is not complete, since the influence of the pollen parent is seen in the ratio of width to length of the cotyledons.

3. The resemblance of the cotyledons of the $F_1$ hybrids to the seed or female parent is maintained in both form and size to full maturity.

4. Matrocliny does not extend to the true leaves in these hybrids. The third, fourth, and fifth leaves are intermediate and identical in reciprocals. Mature plants are identical in reciprocal hybrids of this combination.

5. This is the only instance of matrocliny in *Digitalis* species hybrids observed by the writer.

6. It is impossible to continue the study of cotyledon characters in these hybrids to the $F_2$ and subsequent generations, due to the extreme sterility of *Digitalis* species hybrids. Other plant forms might furnish suitable material for a more extended treatment of the topic.

7. The explanation of the causes of matrocliny in the cotyledons of $F_1$ hybrids between certain *Digitalis* species is difficult. The situation scarcely parallels cases in the lower animal forms. The most probable explanation is the relation of the purely maternal seed coats to the developing embryo. The subjection of the embryo to particular pressures in the different species may explain the form of the cotyledons. The size of the cotyledon may be in proportion to the size of the seed, which differs in the various species.

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# THE "SPRUCE BUDWORM" BIOCOENOSE

## I. FROST RINGS AS INDICATORS OF THE CHRONOLOGY OF SPECIFIC BIOLOGICAL EVENTS

### I. W. Bailey

### (WITH PLATES IX–XI)

### Introduction

Analyses of the growth rings in stems of spruce and fir balsam have proved of considerable significance in studying the disastrous activities of the budworm, *Cacoecia fumiferana*, in the coniferous forests of Maine and eastern Canada. Such stem analyses, for example, have provided the most reliable method of dating the advent of serious infestations in various forest areas. In addition, they have afforded useful criteria in estimating the quantitative effects of varying intensities and durations of defoliation upon the growth and productivity of trees in sample plots.

As might naturally be expected, defoliation by the budworm leads to the formation of more or less abnormal growth layers in the secondary xylem. In the apical portions of the stem cambial activity usually is reduced, even during the first year of feeding, but some wood tends to be formed during each succeeding growing season until the tree dies or recovers. On the contrary, in the basal portions of the stem of seriously defoliated trees the cambium frequently forms an unusually wide ring during the first year, but subsequently may become inactive during one or more growing seasons. Under favorable circumstances, the patterns of abnormal rings are clearly differentiated. Thus, by comparing series of disks from the stems of many trees, and by rejecting all doubtful specimens, CRAIGHEAD (6) has succeeded in tracing the chronology of successive outbreaks of the budworm in eastern Canada.

In conducting more detailed investigations in specific localities, numerous difficulties are encountered in dating the growth layers in certain trees. Owing to variations in the width of annual rings, due to climatic and other environmental factors, it frequently is difficult to determine whether a particular growth layer was formed

during, prior to, or subsequent to the first feeding of the budworm. For example, in figs. 1 and 6 the narrower rings are undoubtedly due to defoliation, but it is not evident which of the three subtending growth layers was formed during the first year of feeding. Each is slightly narrower than the ring which subtends it, indicating a reduction of cambial activity, which, however, may be due to some other factor than defoliation. It is not possible to assign specific dates to these rings by counting backward from the outermost (1922) ring, since one or more of the narrower growth layers may be "false" rings. Moreover, it is not possible to obviate the uncertainty by tracing the rings downward into the basal portions of the stem, for the disappearance of one or more growth layers in basal sections (fig. 2) may be due to arrested cambial activity rather than to the presence of false rings in the terminal sections. It is essential in such cases to obtain some means of accurately dating one of the inner growth layers.

At the request of the Entomological Branch of the Canadian Department of Agriculture, the writer has devoted some attention to the investigation of certain structural abnormalities which are significant in detecting the presence of false rings and in dating specific growth layers. The abnormalities were noted by CRAIGHEAD, and, since they appeared to be more or less closely correlated with the attacks of the budworm, were sent by him to the writer for detailed microscopic examination. They are of two distinct types, dark, reddish brown zones of varying widths, and yellowish brown rings of a somewhat "corky" appearance. The former (fig. 16) are zonal aggregations of "resin" cells and "resin" cysts, which are induced by the feeding of the budworm, as will be shown in the next paper of this series. The latter (figs. 1, 3, 9, 12, 13) are layers of compressed and distorted tissue and will be discussed in the following pages.

### Structure of frost rings in fir balsam

As shown in fig. 12, the rings of distorted tissue consist in part of crumpled tracheids and in part of crushed, undifferentiated, centripetal derivatives of the cambium. In the zone of compression, the rays are laterally displaced or buckled, and are inflated by an enlargement of their constituent cells. Such abnormalities are not

produced by mere defoliation, but are due primarily to mechanical rather than to nutritional disturbances. That they are not induced by the feeding of the budworm is shown also by their occurrence in growth layers formed many years prior to the advent of the insect. They are typical effects of severe frosts upon young, actively growing shoots.

CASPARY (1) demonstrated that contractions induced by low temperatures vary considerably in different tissues. As suggested by SORAUER (5), the greater peripheral contraction of the external tissues during the duration of a frost compresses the delicate cells in the cambial regions, which tend to become deformed. Furthermore, excessive tangential shrinkage in the zone of compression leads to the production of radial cracks or clefts (fig. 13). When the frost terminates and the tissues expand, a normal equilibrium of forces is not restored. Owing to more or less permanent deformations of the tissues, the radial and tangential stresses in the cambial zone are subnormal. This results in an abnormal enlargement and division of the surviving cells, which crowd toward areas of lessened radial and tangential resistance, and thus occlude many of the clefts in the zone of compression. The rays expand tangentially (figs. 15, 17), and the intervening cells frequently tend to differentiate into an irregular short celled parenchyma (figs. 13, 15). As growth continues the stresses are equalized, and the cells assume a normal form and radial seriation.

Although the abnormalities produced by low temperatures are fundamentally similar, their structural details vary in different species of plants (figs. 6–17), and in different representatives of a single species, depending upon the severity and duration of the frosts and the dates of their occurrence (figs. 12, 13, 16). The most constant and characteristic histological features of the frost injuries in fir balsam are the buckling and lateral enlargement of the rays, and the more or less pronounced distortion of the intervening cells; conspicuous radial clefts are not invariably present. The transitions between the zones of compressed tissue and the subsequently formed normal secondary xylem usually are abrupt (figs. 12, 16). Broad intermediate zones of traumatic parenchyma, emphasized by HARTIG (2), SORAUER (5), MIX (3), and others in their investigations of frost injuries, are of relatively infrequent occurrence. In young,

vigorous, actively growing shoots, having wide zones of cambial derivatives in various stages of differentiation, severe frosts tend to produce clearly defined, concentric layers of compressed tissue (fig. 12). There is an inner, broad zone of crumpled, unlignified tracheids, and an outer, narrower zone of crushed and more or less discolored, undifferentiated cells. In the case of milder frosts and of less rapidly growing shoots, these compressed cells may be reduced in number (fig. 16), or may be sporadically distributed, and thus fail to exhibit a striking zonal continuity.

### Distribution of frost rings in fir balsam

The frost rings in stems of fir balsam examined by the writer are confined to the 6–8 innermost growth layers, that is, those in relatively close proximity to the pith. In other words, the stem at any particular level remains susceptible to injury during the first 6–8 seasons of cambial activity. Owing to the fact that each zone of cambial derivatives jackets the previously formed ones and extends beyond them in the apical portion of the stem, the successive frost injuries occur at higher and higher levels. Their longitudinal extension varies in different trees and in different portions of a single cauline axis. This is due in part to fluctuations in the rate of elongation of the terminal shoot. The intervals between the growing points and the levels at which the secondary tissues lose their susceptibility to frost injuries are lengthened in rapidly elongating shoots and are shortened in slowly growing ones.

It is significant in this connection, however, that cambial activity does not always begin simultaneously, or progress at a uniform rate in all trees, or even in all portions of one stem. The condition of the tissues at the dates upon which low temperatures occur must have a more or less marked effect, therefore, not only upon the detailed structure of the resulting frost rings, but also upon their distribution in specific trees or growth layers. For example, the effects of frosts occurring at the beginning of the growing season may be curtailed by cambial inactivity in portions of stems which are not immune to later frosts. The most extensive and clearly defined injuries are those produced by unusually severe late frosts during periods of general, accelerated secondary growth.

As noted by RHOADS (4) in his investigation of various western

Coniferae, the position of frost rings in transverse sections of stems varies considerably in different growth layers. They commonly are located in the first formed portion of these layers, but may occur at times in their median portions, or rarely even upon their outer margins. Such variations in the location of the injuries are obviously determined by fluctuations in the date of the occurrence of low temperatures during different growing seasons. In certain cases, two or even three frost rings (fig. 13) may be present in a single growth layer.

### Significance of frost rings in dating specific growth layers

In view of the fact that frost injuries may readily be distinguished under a hand lens by anyone familiar with their peculiar color and irregular contour, it occurred to the writer that they might prove to be of considerable significance in cross correlating homologous layers in different trees, and thus in accurately dating the growth rings in doubtful cases. An examination of fir balsams from CRAIGHEAD's "Fief St. Claire" and "Saguenay" sample plots in south central Quebec indicated that such is at times the case, and justified a series of more detailed investigations at Long Lake, western Quebec, and near Bathurst, northern New Brunswick.

In each of these regions, the injuries induced by certain specific frosts (unusually severe late frosts occurring during periods of general acceleration of cambial activity) are widely distributed in the vegetation. By sectioning the stems of fir balsams at appropriate levels, it is possible to locate these injuries and to use them as bases in verifying the chronology of growth layers formed during and subsequent to the feeding of the budworm. Most of the fir balsams in the St. Claire and Saguenay sample plots are characterized by having a more or less conspicuous frost injury in the upper extension of their 1910 ring, and those in the Long Lake and Bathurst sample plots by having similar injuries respectively in their 1918 and 1914 growth layers. In south central Quebec the first feeding of the budworm occurred in 1911, at Bathurst in 1914, and at Long Lake in 1918.

As previously stated, it frequently is difficult to differentiate the growth layers formed during the first year of feeding in such stems as those illustrated in figs. 1–6, since false rings may be present in the apical sections and one or more rings may be missing in the

basal sections. The position of the frost injuries in the apical sections, however, may be utilized in obviating such uncertainties as these. Fig. 1 shows the sequence of rings in the terminal portion of a balsam from Fief St. Claire. That the next to the innermost layer is the 1910 ring is indicated by the zone of crushed and distorted tissue near its inner margin. Thus the succeeding layer must have been formed during the first year of feeding of the budworm, that is, 1911. Furthermore, there are eleven intervening layers between the 1910 ring and the outermost or 1922 ring; one for each of the 1911–1921 growing seasons. It is evident, accordingly, that none of the narrower growth layers are false rings. Having dated the rings in the apical portion of the stem, it is possible to trace them downward into its basal portion. The innermost layer in fig. 2 is the basal extension of the 1910 ring. There are only nine succeeding layers, the outermost of which is the 1922 ring. In other words, the cambium remained inactive at the base of the stem during three growing seasons of the 1913–1918 period. In fig. 6, an apical section of a balsam from Bathurst, the position of the 1914 ring is determined by a narrow zone of crumpled and inflated ray tissue. This growth layer, which was formed during the first year of feeding of the budworm, is separated from the outermost (1922) ring by eight intervening rings of varying widths. One of these layers must be a false ring, since there are but seven growing seasons between 1914 and 1922. Under higher magnification, it may be demonstrated that the 1917 layer is divided into two rings by a narrow zone of traumatic "resin" cells, which simulates the outer boundary of a true growth layer. Figs. 4 and 5 are sections of the stem of a balsam from Long Lake. In the section from the upper portion of the stem, there are two wide inner layers and what appear under a hand lens to be four narrow outer layers. The narrower of the two inner layers was originally considered to have been formed during 1918, the first year of feeding of the budworm at Long Lake. In the basal section, the two narrow outer layers are subtended by an unusually wide ring. The enlargement of this ring was interpreted as due to accelerated cambial activity during the first year of defoliation, 1918. Subsequently an additional section was cut at a higher level than that shown in fig. 4. In this section (fig. 3) there are five narrow outer rings. If none of these layers is a false ring, the innermost ring in

fig. 4, which is a basal extension of the corresponding ring in fig. 3, must be the 1918 growth layer. That such is indeed the case is indicated by the position of the frost injury in fig. 3. The 1921 ring in fig. 4 is so tenuous as to be indistinguishable under a hand lens. Furthermore, the wide ring in fig. 5 is not a basal extension of the outermost of the two wide layers in fig. 4, but of the succeeding (1918) ring.

It should be emphasized in conclusion that, in utilizing frost injuries as a means of dating the growth rings in the trees of a specific region, it is essential to determine whether the effects of particular frosts are sufficiently characteristic and widely distributed in the vegetation to justify their use as indicators in stem analyses of individual trees. This involves the examination of very considerable material. In the case of relatively recent outbreaks of the budworm, such as those with which we are at present concerned, the significant injuries fortunately are those which occur in young plants and in the slender terminal portions of old stems. The task of tracing their distribution is not particularly laborious or difficult, therefore, since it does not involve the dissection and detailed examination of the more robust portions of old stems. Furthermore, it is of interest in this connection that the effects of late frosts are visible in the stems of dicotyledons (figs. 7, 8, 10, 11, 14, 15, 17). It is possible to make a preliminary survey of the distribution of frost injuries in such commercially worthless plants as *Alnus, Corylus, Salix, Prunus, Sorbus*, etc., and thus reduce the number of potentially valuable young balsams that must be felled and examined.

A reconnaissance of this character proved to be particularly valuable at Long Lake, western Quebec, a region where late frosts are of almost annual occurrence. An examination of a large number of stems of various dicotyledons revealed a characteristic pattern of frost injuries. In other words, the frost rings formed during the 1913–1915, 1918, and 1922 growing seasons proved to be very clearly differentiated and widely distributed in the dicotyledonous vegetation. That more or less severe frosts occurred during the intervening growing seasons was indicated by the presence of additional frost injuries in the stems of the more susceptible species, for example *Sorbus* (fig. 7), but the effects of these frosts were found to be of extremely sporadic occurrence in the less susceptible dicotyledons.

Even in the case of the mountain ash, the 1913–1915, 1918, and 1922 frost rings could readily be distinguished from the intervening frost injuries by characteristic differences in their position, form, and severity (fig. 7). It was subsequently determined that similar patterns of frost injuries occurred in stems of fir balsams from CRAIG-HEAD's sample plots, and, therefore, it was possible to utilize the conspicuous 1913–1915 and 1918 frost rings as indicators in verifying the chronology of the outermost growth layers.

## Summary and conclusions

1. In studying the more or less abnormal growth layers in the stems of conifers defoliated by the spruce budworm, it is difficult in many cases to determine which rings were formed during specific growing seasons. This is due to the frequent omission of one or more growth layers, particularly in the basal portions of the stem, and to the occurrence of structures resembling false rings.

2. A reconnaissance of frost rings in the vegetation of various forest areas in eastern Canada indicates that the injuries produced by certain frosts are widely distributed in the stems of fir balsam, alder, hazel, birch, willow, poplar, cherry, mountain ash, etc. They afford a reliable means of cross correlating homologous growth layers in different trees, and, therefore, of accurately dating specific growth layers in fir balsams defoliated by the budworm.

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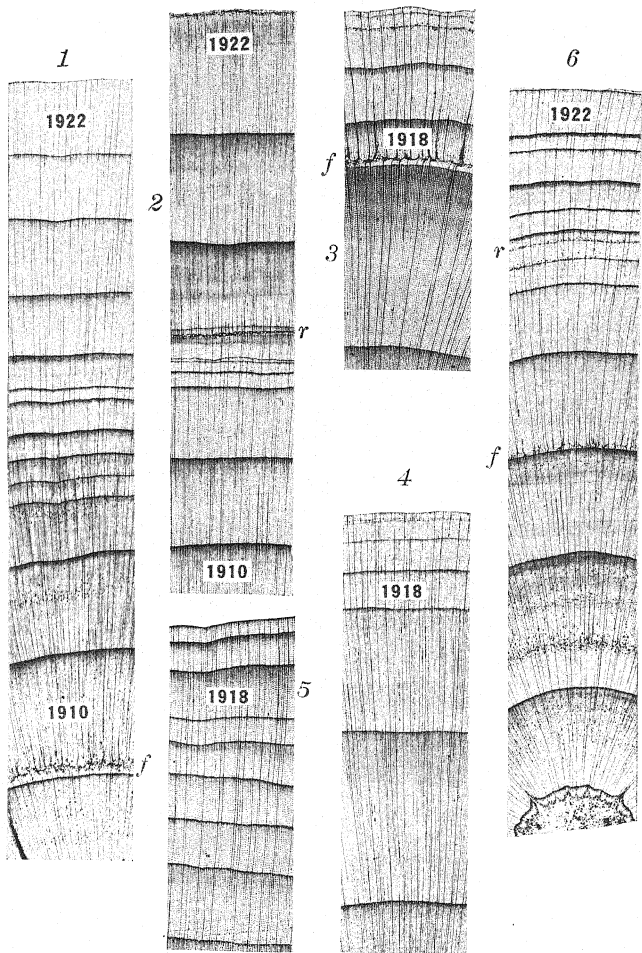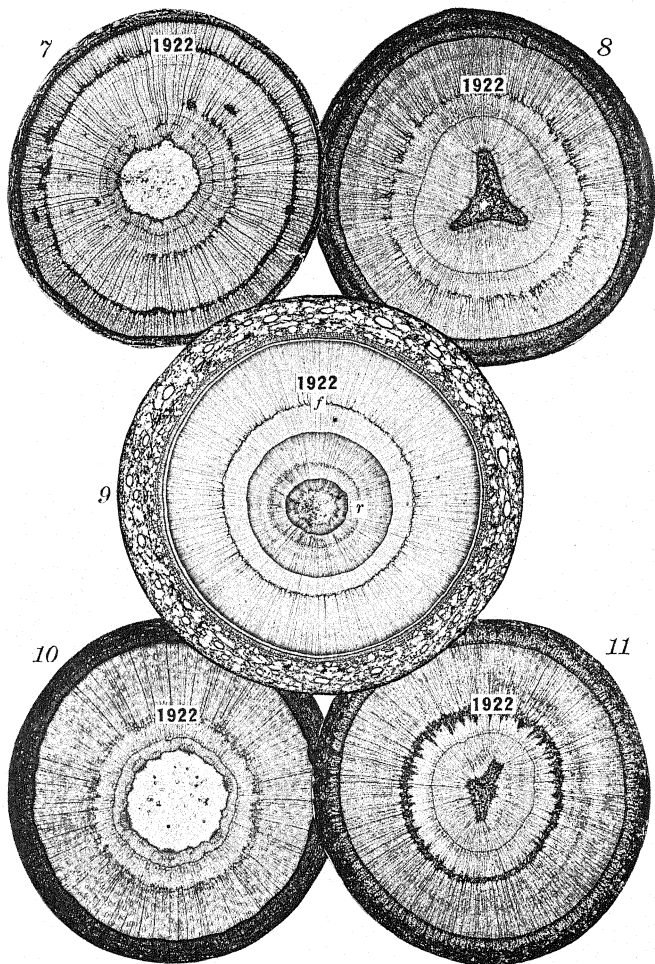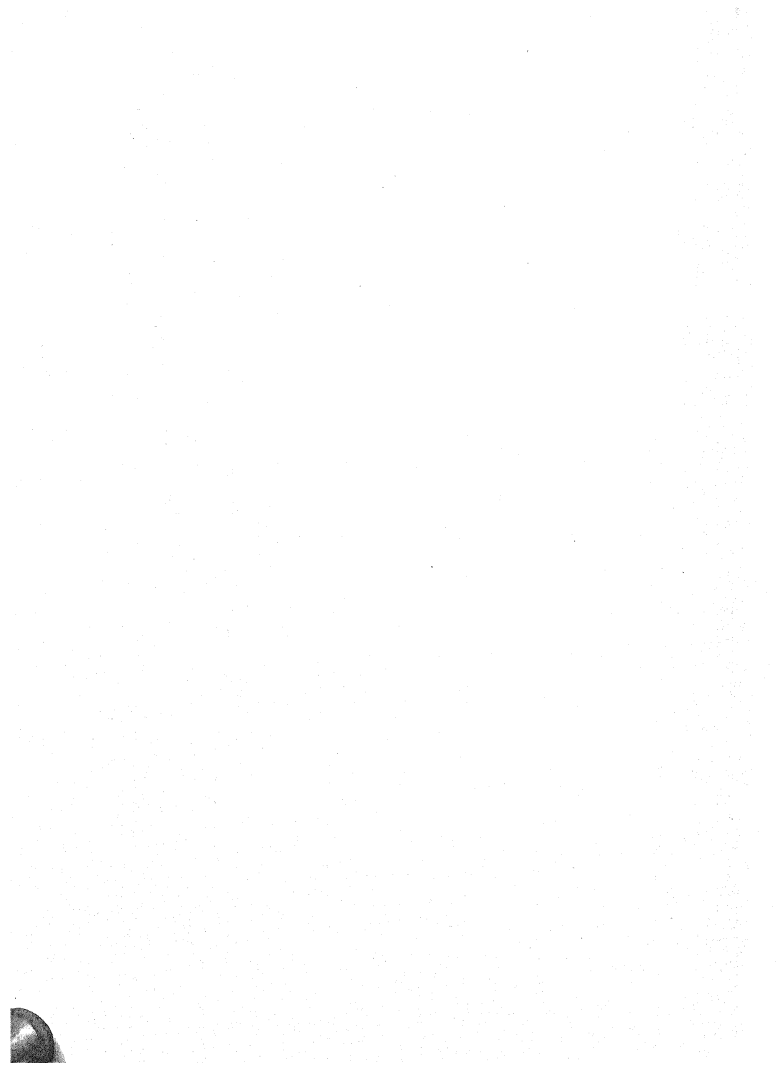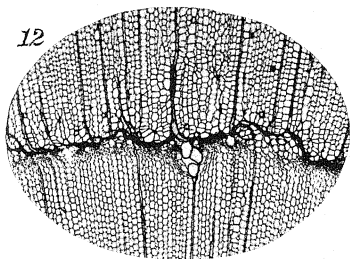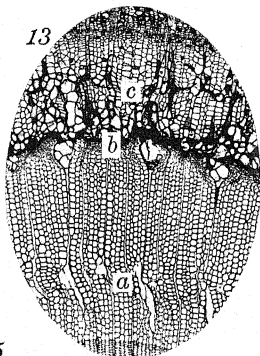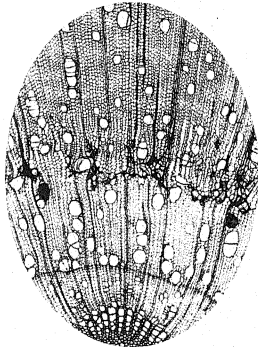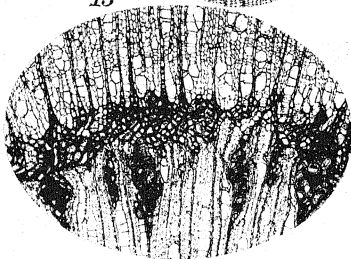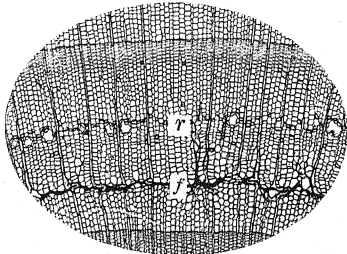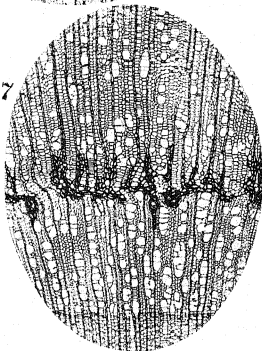
## DESCRIPTION OF PLATES IX–XI

Fig. 1.—*Abies balsamea:* transverse section of terminal portion of old stem, showing pattern of abnormal growth layers in tree which recovered from attacks of budworm; *f*, frost injury; first feeding of budworm in 1911; ×7.

Fig. 2.—Same: transverse section of basal portion of same stem, showing omission of three growth rings during 1913–1918 growing seasons; *r*, arc of traumatic "resin" cysts induced by feeding of *Pissodes dubius;* ×9.

Fig. 3.—Same: transverse section of terminal portion of old stem, showing pattern of abnormal growth layers in dying tree; *f*, frost injury; first feeding of budworm in 1918; ×15.

Fig. 4.—Same: transverse section of same stem, cut at slightly lower level than that shown in fig. 3; ×10.

Fig. 5.—Same: transverse section of basal portion of same tree, showing omission of 1921 and 1922 growth layers and marked acceleration of cambial activity during 1918; ×10.

Fig. 6.—Same: transverse section of terminal portion of old stem, showing pattern of abnormal growth layers; *f*, frost ring; *r*, ring of traumatic "resin" cells, simulating outer boundary of growth layer; first feeding of budworm in 1914; ×7.

Fig. 7.—*Sorbus americana* (Marsh.) DC.: transverse section of terminal shoot of young plant, showing effects of 1920, 1921, and 1922 frosts; ×12.

Fig. 8.—*Alnus incana* (L.) Moench.: transverse section of stem of young plant, showing characteristic 1922 frost injury; ×11.

Fig. 9.—*Abies balsamea:* transverse section of terminal shoot of young plant, showing characteristic 1922 frost injury; *f*, frost injury; *r*, ring of traumatic "resin" tissue; ×7.

Fig. 10.—*Salix* sp.: transverse section of young stem, showing characteristic 1922 frost injury; ×11.

Fig. 11.—*Betula alba* L. var. *papyrifera* (Marsh.) Spach.: transverse section of terminal shoot of young plant, showing characteristic 1922 frost injury; ×8.

Fig. 12.—*Abies balsamea:* portion of fig. 9 more highly magnified, showing zones of compressed tissue and buckling of rays; ×46.

Fig. 13.—Same: transverse section of terminal shoot of young plant, showing the effects of three successive frosts; at *a* radial clefts and slight distortion of tracheids; at *b* zones of compressed tissue, "inflated rays," and zone of traumatic parenchyma; at *c* narrow, irregular zone of compression; ×50.

Fig. 14.—*Salix* sp.: portion of fig. 10 more highly magnified, showing zone of compression and distorted tissue; ×45.

Fig. 15.—*Betula alba* var. *papyrifera:* portion of fig. 11 more highly magnified, showing zone of compression, "inflated rays," and outer zone of traumatic parenchyma; ×45.

Fig. 16.—*Abies balsamea:* transverse section of wood, showing frost ring at *f*, and zone of traumatic "resin" tissue at *r;* ×45.

Fig. 17.—*Alnus incana:* portion of fig. 8 more highly magnified, showing buckling and enlargement of rays; ×50.

# HYDROLYTIC ENZYMES IN PHORMIDIUM LAMINOSUM

## OLGA LAKELA

Little work has been done on the occurrence of enzymes in the blue-green algae, in comparison with the extensive research work done on the enzymes of bacteria, fungi, and higher plants. According to GREEN (2), lipases occur in oil bearing seeds. CAMUS (1) reports them in higher and lower fungi, and they also have been found in various bacteria.

Amylases are widely distributed in the plant kingdom. As early as 1814 KIRCHOW observed the decomposition of starch in germinating barley. The isolation of the enzyme in concentrated form was accomplished in 1853 by PAYEN and PERSOZ, who named it diastase. Since then many amyloclastic enzymes have been detected in many plant products. Invertase occurs in many fruits, in germinating pollen grains, in some fungi, and in bacteria. VINES (9) discovered proteolytic enzymes in seeds, leaves, in certain juicy fruits, and in insectivorous plants. They have also been observed in fungi and bacteria.

It has commonly been believed that certain of the enzymes occur universally (3), and that the metabolic processes of a living organism are controlled by the presence or the activity of these enzymes. The enzymes present in an organism may determine the substrate in which it can grow. The presence of these common biological catalysts, however, is not necessary for the vital reactions if the syntheses or hydrolyses which they catalyse under ordinary conditions can be brought about by physical or chemical conditions such as high temperature or acidity.

This study was undertaken to demonstrate the importance of enzymatic action in the life processes of an alga which lives above the maximal temperature for most enzymes. The investigation showed the absence of some of the most common enzymes in plants. A chemical analysis of the algal material was also undertaken, to determine what substances were present in the metabolism of which enzymatic activity would be of importance. It is difficult to perceive

in the scale of evolution how organisms could start with a full complement of enzymes, since in many cases these have been shown to be complex and labile substances. It would seem more logical to assume that the extremely primitive organisms might equally well be dependent upon the rates of reactions, which are determined by external conditions such as the temperature, rather than dependent upon the complex and delicate catalysts such as many enzymes are known to be. The thermal algae are known to be very primitive with regard to morphological organization and in their physiological processes. Many of them live under conditions for nutrition such as may have prevailed at the time of the origin of living things. Their adaptation to temperature as high as 89° C. indicates that their ecological requirements are much different from those of many higher plants in this regard.

The collections of algae used in this study were made from Hymen Terrace Spring at Yellowstone National Park by Dr. R. B. HARVEY in June, 1923. The temperature of the spring water at the time of collection registered 73°–74° C. The material was preserved either with toluol or with 80 per cent alcohol. The alga was identified as *Phormidium laminosum* (Ag.) as described by TILDEN (**5, 6**).

## Diastase and invertase

For a determination of diastase and invertase in this algal material, one per cent solutions of sugar and of starch were prepared. The specimen preserved with toluol was used in tests of enzymatic activity. Since autolysis may have occurred in the material, the enzymatic activity of the preserving fluid, as well as the material itself, was determined. The mass of algal filaments with its mineral contents was well ground in a mortar, and a suspension in distilled water was prepared. To estimate the dry weight of the material taken for each experiment, an aliquot of the suspension was filtered, dried, and weighed. The tests were set up as follows:

SERIES I

1. 25 cc. starch 1 per cent+10 cc. filtered extract
2. 25 cc. starch 1 per cent+10 cc. suspension
3. 25 cc. sucrose 1 per cent+10 cc. filtered extract
4. 25 cc. sucrose 1 per cent+10 cc. suspension
5. 25 cc. distilled $H_2O$+10 cc. filtered extract
6. 25 cc. distilled $H_2O$+10 cc. suspension

The first four samples were left in an incubator for 30 minutes at 48° C. The values of 5 and 6 were determined directly. In determining the values for these solutions, the method described by THATCHER (7, 8) was used. For measuring the quantity of reducing sugars, PETER'S (4) iodide method was found to give the most satisfactory results.

The values of Fehling's solution, the starch, and sugar substrate used as controls checked with one another. In the incubated solutions some hydrolysis occurred. Sample 2, which contained the starch substrate, and the suspension produced 26.40 mg. of dextrose; however, this result is small enough to lie within the range of experimental error.

Further trials were carried out at a temperature of 74° C. The solutions were identical in composition with those of the first series, and the same methods were applied. Most reduction occurred again in sample 2, as in the identical one of the first series. This time the dextrose value was 9.20 mg., which was less than the value obtained at a lower temperature. The same was true of the other samples. If, therefore, the reduction in these experiments is due to the enzymatic activity, the activity decreases at the temperature of 74° C. The plant grows at a temperature of 74° C., however, and its enzymes, if present, might be expected to act at this temperature.

In the third series of trials, identical samples were subjected to a temperature of 74° C., for twenty-four hours. The titration values for the controls and the digested samples were identical. Obviously in this series of trials the conditions for enzymatic activity were most favorable, but the results do not indicate any, so that the absence of diastase and invertase from *Phormidium* seems definitely established.

### Proteose activity

Tests for tryptophane were determined according to the directions in A.O.A.C. Methods of Analysis (11). Experiments with casein substrate showed that *Phormidium* does not contain a casein splitting enzyme. Further trials were made to discover whether or not the algal material contains an enzyme capable of hydrolyzing its own protein. The plant material preserved in toluol was allowed to act on protein prepared from the material which had been preserved in alcohol. One set of samples was incubated at 48° C. for twenty-

four hours, another set at 74° C. for the same length of time. The test for tryptophane in all the samples was negative. Evidently some reaction such as that of VAN SLYKE will need to be used to determine proteose activity, since tryptophane seems deficient in the protein; but for such an experiment a sufficient quantity of the material was not available.

Lipase activity was determined according to the directions by SHARP and MORROW (12). In the digested samples a slight increase in acidity occurred, due to the presence of free fatty acids formed by lipase activity. To test the heat resistance of lipase, a series of experiments was carried out at temperatures from 74° to 95° C. Some interfering reaction, however, induced by high temperature, prevented the formation of free fatty acids, causing decreasing acidity. Nothing could be concluded as to the heat resistance of this enzyme.

Glycogen is a common carbohydrate in the blue-green algae. Since *Phormidium* belongs to this group, it was subjected to a test for a glycolytic enzyme. A substrate of 0.4 per cent glycogen was prepared, and the experiments were carried out according to the methods given under diastase and invertase activity. The dextrose value obtained was 9.55 mg. If this small amount of reduction is not due to experimental error, the action may be said to be due to a glycolytic enzyme.

### Nutritive substance

A portion of the collection preserved in alcohol was used in tests for nutritive substances. The official methods (11) were applied. Reducing sugars or sucrose were not present. Organic nitrogen was determined by Kjeldahl's method. The protein represented 2.6 per cent on the basis of ash free substance. Free amino acids were found present in the material.

On acid hydrolysis the alcohol insoluble residue yielded 1.35 per cent of starch. It is questionable whether this value represents starch. Other polysaccharides probably in the wall may yield dextrose on acid hydrolysis. A microchemical study failed to show starch grains in the algal material. Glycogen was found to represent 0.5 per cent of the material on a dry weight basis. Pentosans were not present.

## Summary

1. The alga growing in Hymen Terrace Spring at 74° C. was identified as *Phormidium laminosum* (Ag.).

2. The plant material contains 53 per cent of ash on dry weight basis.

3. The absence of diastase and invertase from *Phormidium* was definitely established.

4. *Phormidium* does not contain a casein splitting enzyme; the plant protein does not contain tryptophane in detectable quantity.

5. Lipase activity was shown at 48° C.; at 74°–75° C. its activity could not be determined, on account of some interfering reaction which prevented the determination of free fatty acid production.

6. Glycogen splitting enzyme was probably present.

7. *Phormidium* does not contain sugars, starch, or pentosans in detectable quantity.

8. Proteins, amino nitrogen, and glycogen were present.

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# ENLARGED BASES IN FRAXINUS NIGRA IN MICHIGAN[1]

### FRANK C. GATES AND C. O. ERLANSON

#### (WITH TWO FIGURES)

In the northern part of the lower peninsula of Michigan, the lowlands in general are bogs or swamps in which *Thuja occidentalis* L. largely predominate in the natural vegetation. Following lumbering or burning, the new forest, especially along streams, frequently contained a high percentage of *Fraxinus nigra* Marsh. In extreme cases the groves might contain little but black ash. This is a perfectly normal habitat for this tree, as it grows readily in wet places from streams to undrained bogs. The striking thing about these trees growing along certain streams or in their immediate vicinity, however, is the base, which is enlarged very much more than seems to be necessary from any standpoint under consideration. These trees grow somewhat close together, so that they protect one another from the wind. Furthermore, the trees are deciduous and therefore less exposed to the severity of winter. None of the other trees present in such habitats have the striking enlargements of their bases.

The best examples of this enlargement occur in the delta of Maple River, just southwest of Colonial Point (fig. 1). The trees are well developed, approximately of the same age, and about 6–8 inches in diameter above the base. The diameter of the base itself is about 13–15 inches. The seedling trees that are now appearing in this area are usually red maple or white cedar rather than black ash. A second area in which the enlarged bases may be found is that at the southwestern corner of Smith's bog, in which, however, there are only a few such trees.

The root system of these trees is quite shallow, as is normally

found in the case of trees growing in water. The changes in lake level are not sufficient to cover more than about one-third of the height of the enlarged bases. Frequently, after the trees have grown to the point of casting considerable shade, the ground plants beneath die out, and the surface soil breaks up and washes away, so that, in places in the immediate vicinity of the streams, one will find these trees apparently perched on top of the bottom, rather than beneath the soil under the water.
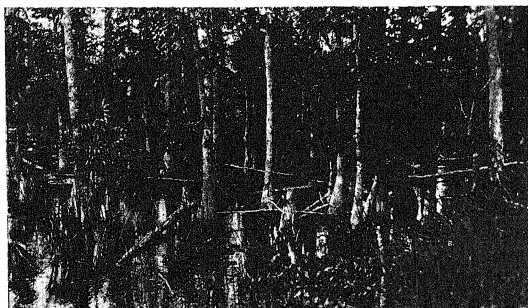


FIG. 1.—View of deltal region of Maple River, showing enlarged bases of *Fraxinus nigra*, August 8, 1921.

The general appearance of the above-ground parts of these ash trees is much the same as that of black ash trees growing in bogs. They differ only in that the branches are inclined to be rather short, although spreading widely from the trunk. The upper parts of the trees are frequently dead. At a distance the woods appear to be full of dead trees, with a growth of younger trees coming on but not quite topping the dead stems. This appearance, however, soon gives way to the true state of affairs as one enters the woods. The green shoots for the most part come from a little below the tops of the trees, and not from along the trunk in the vicinity of the base, or from younger trees. From the swollen base to the crown there is very little change in the thickness of the trunk, but at the top it narrows to an abrupt
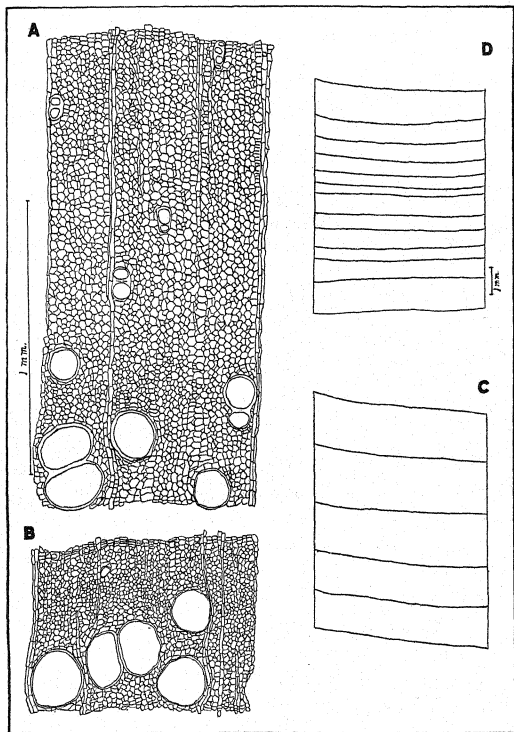
Fig. 2.—Sections of black ash trunk drawn with aid of projection apparatus: A, B, transverse sections of typical year's growth, A, taken from swollen base, B from normal trunk; C, D, transverse sections showing annual rings, C in swollen base, D in normal trunk (scale for A and B on left, and for C and D on right).

irregular conical shape. The swollen bases of the ash trees make the area very suggestive of swamps in the southern states, and not what one would expect to find in the north. Nothing resembling cypress knees, however, was discovered in the black ash groves.

Sections were made in three planes, 15 $\mu$ thick, from various points along the radius of the enlarged base. Sections were also made from the normal bole farther up on the tree, at corresponding positions to those taken below, so that comparisons could be made (fig. 2). Sections made at the center of the trunk show that for about the first thirty years the tree was normal throughout its trunk. The annual rings of the swollen part then gradually become farther apart up to a certain width, which is maintained, with small fluctuations, to the cambium. A distance of 3 cm. on the radius of the swollen part covers thirteen annual rings, whereas the same distance on the radius of the corresponding normal part covers thirty-three annual rings.

The average diameters of the elements in the swollen part do not differ from those in the normal part, nor are they in any way abnormal in shape. The number of large spring vessels, in transverse section of any fixed area of the swollen part, is the same as the corresponding area in the normal part. There is no increase in the number of vessels in the swollen part. The increase in the width of the swollen base is due to the great increase in the number of cells of the summer wood.

### Summary

Specimens of *Fraxinus nigra* Marsh growing in permanently water covered areas adjacent to certain streams in Cheboygan County, Michigan, have a very conspicuously enlarged base, in most cases nearly twice that of the diameter of the trunk above.

The swollen base is due, mechanically, to the great increase in the number of cells of the summer wood of each year's growth, there being about twice as much growth in the swollen part as in the normal part. The individual cells of the swollen part are not abnormal either in size or shape.

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# BRIEFER ARTICLES

## MARK ALFRED CARLETON

### (WITH PORTRAIT)

MARK ALFRED CARLETON died April 26, 1925, in Paita, Peru, from an attack of malaria. Although widely known by his work in vegetable pathology, CARLETON established his greatest record by his work on the introduction of new varieties of cereals into this country, and his death is a severe loss to American agriculture. He was born near Jerusalem in Monroe County, Ohio, in 1866. When he was ten years of age, his parents moved to a farm in Cloud County, Kansas. His early education was obtained in the rural schools of Ohio and Kansas. In 1884 he entered the Kansas Agricultural College at Manhattan, where he completed his course and also a year of special work in biology and chemistry, graduating with the degree of Bachelor of Science in 1887. During 1890–1891 he was Professor of Natural History in Garfield University at Wichita, Kansas; during 1891 and 1892 he taught natural history in Wichita University; and during 1892–1893 he took a postgraduate course in botany and horticulture at the Kansas Agricultural College, receiving the degree of Master of Science. During 1893 he was Assistant Botanist at the Kansas Experiment Station, giving special attention to plant pathology and particularly to cereal rusts.

In 1894 CARLETON began his service in the United States Department

of Agriculture, by appointment as Assistant Pathologist, his time being devoted chiefly to the study of cereal diseases. In 1901 he was appointed Cerealist in Charge of Grain Investigations in the Bureau of Plant Industry, a position which he held until May 8, 1918. During 1898 and 1899 he was an Agricultural Explorer in eastern Europe and Siberia, in search of rust-resistant and drought-resistant cereals. In 1900 he was Expert in Charge of the Grain Exhibit of the United States at the Paris Exposition, and in the same year he made another trip to eastern Europe in search of hardy cereals, and to increase the supply of those originally obtained.

Before entering the Department of Agriculture, CARLETON had published some reports on rusts of grains, some lists of Kansas parasitic fungi, and some observations on various flowering plants of Oklahoma, etc. It was under the auspices of the Department of Agriculture, however, that he published his great work in plant pathology, establishing the physiological relationships of nearly all the cereal rusts of this country, demonstrating the distinctness of the different forms of the same species adapted to the same host, and showing that durum wheats, emmer, einkorns, and some other wheats are more or less rust-resistant.

Having visited Russia and Siberia, CARLETON had acquired a thorough knowledge of the various cereals cultivated in these countries. Being a very keen observer, he selected a number of varieties suitable for cultivation in this country, and their introduction proved a great success. According to the Proceedings of the American Society of Agronomy (1910), the introduction of durum wheat means an annual yield of 60,000,000 bushels; the introduction of Swedish Select oat furnishes 40,000,000 bushels of the annual oat crop, etc. The introduction and establishment of these cereals was a very difficult task, and CARLETON had to make many experiments at the experiment farms in various parts of the country to test their hardiness and disease resistance. His very comprehensive paper, *The basis for the improvement of American wheats*, gives an idea of the magnitude of the work which he accomplished in this direction. It is really remarkable what an immense field CARLETON covered in so short a period as twenty-five years, especially considering his very modest education in natural science. Three years of Latin and one year of Greek constituted his fundamental knowledge of languages, and yet he undertook the difficult task of studying the Russian language before going abroad in search of new varieties of cereals.

After leaving the service of the United States Department of Agriculture in 1918, CARLETON undertook some important work with the Grain

Corporation of America. This was about the close of the World War, and the Grain Corporation of America had some very important economic problems in connection with cereals to handle. In this work he rendered excellent service. Leaving the Grain Corporation, he entered the service of the United Fruit Company, and was sent to Central America to make a study of banana diseases. He had headquarters for some time near Port Limon, and there developed a laboratory and conducted some important field investigations in the selection of disease-resistant bananas. In 1922 he severed his relations with the United Fruit Company and was called to British Honduras for pathological work in connection with another large fruit company of that country. He remained in British Honduras until December, 1924, when he went to Peru in connection with a large project having for its object the production of cotton and other crops in Peru, and was making special studies of cotton diseases at the time of his death.

CARLETON was a self-made man, full of energy and inspiration, and always active, and his keen eye for nature guided him to accomplish what he did. In the history of American botany his name will be recorded as one of the leading pathologists, whose work culminated in a result of such magnitude as to mark an epoch of the greatest importance to American agriculture.—THEO. HOLM, *Clinton, Md.*

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NOTICE

Although the residence of the editor is changed from Chicago to Yonkers, N.Y., for the coming year, he will still have charge of this journal.

All communications and manuscripts should be addressed to **Editorial Office, Room 11A, Botany Building, University of Chicago.**—J. M. C.

# CURRENT LITERATURE

## BOOK REVIEWS

### The cell

It is thirty years since the first edition of WILSON's work on the cell appeared. Four years later a second edition followed, which included considerable revision, especially in the section dealing with the centrosome, and which brought the literature up-to-date. No other book ever did so much to stimulate investigations upon the structure, development, and functions of the cell. This authoritative presentation enabled the younger generation of zoologists to undertake researches which would have been impossible without such aid. Botanists, having no similar presentation in their field, used WILSON's book, and thus obtained some knowledge of the animal cell and its problems. If such a book as SHARP's *Introduction to cytology* had appeared in the nineties, the zoologists of today might have had a better appreciation of the plant cell and its problems.

A new volume by WILSON[1] has now appeared, which is not really a revision of the earlier editions, but a new book, with the number of pages increased from 483 to 1232 and the number of illustrations from 194 to 529. The paper is thin and of such excellent quality that the illustrations are well reproduced, and the book is not unwieldy to handle.

In general, the book is written frankly from the standpoint of the zoological student of cytology and embryology, without pretence of competence to deal with outside fields; but the treatment of plant cells and botanical problems is much more accurate and extensive than in previous editions. While the botanist will still depend upon SHARP, the opinion of a competent zoologist in regard to structures which seem to be identical in plants and animals will always be helpful and suggestive.

WILSON's book is very different from the *General cytology*, recently published by the University of Chicago Press, which was written by thirteen different authors, presenting special aspects of cytology from the standpoints of specialists in different fields. Their work is valuable, but, like many botanical textbooks, lacks unity, and does not give the comprehensive treatment of the subject which was so stimulating in the earlier editions of *The cell in development and inheritance*, and which has been so greatly extended and improved in the present edition.

A glance at the table of contents of the third edition shows numerous topics which were practically unknown in 1900. These newer subjects, especially the

chondriosomes of both plants and animals, are described in detail and thorough-
ly illustrated. The feature which most impresses the older investigator, how-
ever, is the changed point of view in treating subjects like the chromosome, cen-
trosome, fertilization, and embryogeny, showing the great advance of knowl-
edge in this direction during the last few decades.

Throughout the book the treatment shows the perspective and caution
which comes from increasing knowledge of the facts, and this is nowhere more
evident than in descriptions and discussions of chromosomes. Here much more
attention is given to plants than in the previous editions. The presentation is
eminently fair, although it is plain that the author does not agree with the the-
ories of the Gregoire school in regard to the structure of the chromosome. They
do not believe in the existence of the chromomere as a definite entity, but WIL-
SON believes that "such skepticism cannot be maintained in view of the positive
results of recent careful studies." Nevertheless, the reviewer must confess to an
increasing skepticism in regard to the universal existence of chromomeres. If
both theories are correct, so that in some forms the chromosome may consist of
linin and chromomeres, while in other forms it consists of only homogeneous,
vacuolated chromatin, theories of heredity will be more difficult to formulate.

An antithetic alternation of generations in animals, with the egg and three
polar bodies (polocytes) as one generation and the soma as the other, is still
denied; but the discussion, which is intended to prove that an antithetic alter-
nation of generations, like that in plants, does not exist in animals, shows that
the author has studied botanical literature and progressed far beyond his col-
leagues in familiarity with botanical problems. Another advance like this might
give us an account of antithetic alternation of generations in animals, and give
an interpretation of the polar bodies which would seem logical to a botanist. As
the account stands, our viewpoint in regard to alternation of generations in
plants is not yet fully appreciated, as is indicated in the statement that "in
*Fucus vesiculosus* there is only one generation, the diploid, as in animals." We
agree that in *Fucus*, and better still in *Plumbagella*, the situation is the same as in
animals; but the origin and fundamental nature of alternation of generations
in plants demands two generations at the extremes, like *Spirogyra, Fucus,* and
*Plumbagella*, just as in easily recognized intermediates like *Cutleria*, mosses, and
ferns. We agree, absolutely, that there is no genetic relation between alterna-
tion of generations in plants and animals, but we believe there is a case of paral-
lel development such as is seen in the attainment of heterospory by unrelated
phyla of plants.

The treatment of the reduction divisions in both animals and plants will be
particularly helpful to botanists, because they are put into easy contact with the
zoological situation by clear diagrams, in addition to a profusely illustrated text.
In this connection, there is an extended presentation of differentiation among
chromosomes, the relation of chromosomes to the determination of sex, and to
various phases of heredity.

The comprehensive treatment of the embryology of animals should be sup-

gestive to botanists, for the embryology of plants has never been so thoroughly worked out, and what results have been obtained have not been so thoroughly organized.

The glossary will doubtless be welcome, even to zoologists, and for botanists it is indispensable. The double subject and author index is extensive, and has numerous catchwords which will save time in looking up references. A special bibliography is given at the end of each chapter, and in addition there is a general list of literature occupying 55 pages. The number of papers cited was not counted, but was roughly estimated to be about 2,000 citations.

On the whole, the book will stimulate and facilitate botanical as well as zoological research, and the reviewer predicts that for a long time it will hold its place as the standard work on cytology.—C. J. CHAMBERLAIN.

### Colloid chemistry of protoplasm

A very readable discussion of the general and special colloidal constitution and behavior of protoplasm has been prepared by LEPESCHKIN,[2] whose contributions to protoplasmic behavior have won him recognition in this field. The volume is no. 7 of the series of *Monographien aus dem Gesamtgebiet der Physiologie der Pflanzen und der Thiere*. The introductory section deals with the general nature of hydrophobe and hydrophile colloidal state, and the problems of diffusion, osmosis, electrical charge, adsorption, viscosity, surface tension, and changes in the degree of dispersion. It also considers the general colloid chemistry of the proteins and lipoids.

The second section, part I, presents a picture of the general chemistry of protoplasm as a mixture of substances in colloidal state. Consideration of state of aggregation of protoplasm, nucleoplasm, plastids, and fibrillae leads to the conclusion that in the active state, protoplasm and the plastids form a colloidal solution, not a gel, except in the case of nerve and muscular fibrillae. These fibrillae are gel structures. The general colloidal structure of the living material, disperse phase and dispersion medium, receives detailed consideration, and some attention is given to the problems of viscosity, osmotic pressure, and electrical phenomena in the living protoplasm. Reversible and irreversible phenomena are described. With reference to the problem of osmotic pressure LEPESCHKIN finds the membrane idea of osmosis useless. In this connection it is obvious that LEPESCHKIN, like many others, has his attention centered too much upon the non-diffusible elements, which serve only to produce continuing unbalanced distribution of water, and not enough upon the diffusible water which is mainly responsible for the development of pressures, whether a membrane is present or not.

The final section, part II, presents the special features of protoplasmic colloidal behavior, those things in which protoplasm differs from other colloidal

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[2] LEPESCHKIN, W., Kolloidchemie des Protoplasmas. 8vo. pp. xii+228. Berlin: Springer. 1924.

systems. He takes up first the chemical constitution of the dispersion medium
(which is not water, but a substance in which water dissolves), and the disperse
phases of cytoplasm, nucleus, and chromatophores. Then follows a discussion
of the effects of high and low temperature changes, mechanical agencies, desic-
cation, light, and electric currents. The final pages consider the influence of elec-
trolytes, including the neutral salts, salts of trivalent metals, heavy metals, acids
and bases, and of non-electrolytes such as the indifferent narcotics, alcohol, ace-
tone, chloroform, benzol, ether, etc., upon the colloidal state of these phases.
The nature of narcosis receives very brief consideration.

     Those who have kept up with the research will no doubt consider this book
an elementary treatise. It is simply written, and for beginning students of pro-
toplasmic behavior it presents a timely summary of the great advances which
are being made in development of biological theories, and in the understanding
of the conditions and processes of life, through the application of colloidal physics
and chemistry to the investigation of the living substances of plants and animals.
—C. A. SHULL.

### The American oaks

     TRELEASE[3] has published a notable contribution to our knowledge of the
taxonomy of oaks, the result of many years of investigation. His original plan was
to study the species of tropical America, those of the United States having been
sufficiently presented. Finally, however, he included all of the American oaks,
those of the United States being treated in a more summary way than the
others. Naturally the treatment of old segregates and the publication of new
ones will attract the attention of taxonomists interested in a very puzzling genus.
In consequence of the previous taxonomic tangles, the author has attempted to
reorganize the material in a more satisfactory way.

     The broad background of his conclusions was developed by a study of all of
the types, which are reproduced in excellent photographic illustrations, by an
investigation of the past history of oaks, and by a consideration of the develop-
ment of the geographic areas involved. In this way he has been able to unify
the nomenclature of fossil and living species, and to organize the species into
natural groups, indicating in a chart the convergences and affinities among
these groups.

     There is a full description of the characters of the genus and their relative
value in indicating relationships. Abnormalities are also included, as well as a
list of American hybrids. A noteworthy item is the recognition of *Protobalanus*,
as including "the intermediate oaks," and its probable ancestral relationship
to the white and black oak groups. Some idea of the amount of material investi-
gated may be obtained from the fact that 373 species are recognized, in addition
to 32 varieties and 221 forms. For example, under *Q. stellata*, 14 forms and one

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     [3] TRELEASE, W., The American oaks. Mem. Nat. Acad. Sci. 20:pp. 255. *pls. 420*.
1924.

variety are recognized. These numerous species, with their varieties and forms, are further organized into 131 natural groups.

This contribution will certainly be a standard reference in any study of the taxonomy of American oaks.—J. M. C.

### The New England–Acadian shoreline

An attractive volume has recently appeared[4] that deals with the shores of the maritime provinces of Canada and those of New England. While the reviewer is not able to criticize the accuracy of the main physiographic portion of the book, he has found the material interesting, well organized, clearly presented, and well illustrated by means of photographs, maps, and diagrams. It is necessarily of decided interest to ecologists studying the shore vegetation, and more especially because, in addition to describing the physical substrata for shore plants, it presents a rather detailed study of salt marshes. The different theories accounting for the origins of such marshes are discussed, and the theory propounded by MUDGE is favored, that is, that the marsh is usually a record of a progressive subsidence of the land during the time of its formation. There seem to be three rather distinct types of such marshes, designated from the regions of their development: (1) New England type; (2) Fundy type; and (3) Coastal plain type. The first type usually exhibits three distinct, rather broad zones characterized by *Juncus Gerardi*, *Spartina patens*, and *S. glabra* respectively, and is developed upon a substratum consisting largely of salt marsh peat. The second type has much more silt in its substratum, and much narrower strips of vegetation. The third type is rather closely related to the second, but differs somewhat from it in the character of the silt and in the floristic composition of the vegetation. Variations from these types are discussed and many examples are cited. Extensive bibliographies are appended to each chapter.—GEO. D. FULLER.

## MINOR NOTICES

**Phytoplankton of Wisconsin.**—The Wisconsin Geological and Natural History Survey has been conducting an important investigation of the phytoplankton of the inland lakes of Wisconsin. The first publication of the results appeared in 1920, including all plankton algae except desmids and diatoms. The second part has just appeared,[5] presenting the Desmidiaceae. The species are amply described and illustrated by 37 excellent plates, resulting in a notable contribution to our knowledge of this group. The volume presents 22 genera of Desmidiaceae, including 159 species. Much the largest genus is *Staurastrum*, with 59 species, 5 of which are described as new, the only new species in the volume, although there are numerous new varieties. The next largest genera are *Cosmarium* (15 species), *Micrasterias* (15 species), *Euastrum* (11 species),

[4] JOHNSON D., The New England–Acadian shoreline. 8vo. pp. xx+628. *figs. 273.* New York: John Wiley & Sons. 1925. $8.50.

[5] SMITH, G. M., Phytoplankton of the inland lakes of Wisconsin. Part II. Desmidiaceae. Wis. Geol. and Nat. Survey, Bull. 57. pp. 227. *pls. 52–88.* 1925.

*Arthrodermus* (11 species), and *Closterium* (10 species). The remaining 38 species are distributed among 16 genera. This contribution is also issued as a Bulletin of the University of Wisconsin.—J. M. C.

## NOTES FOR STUDENTS

**Taxonomic notes.**—BERGER[6] has made a very critical taxonomic study of *Ribes* and *Grossularia*, including wild species and forms under cultivation, involving numerous hybrids. He recognizes 67 species of *Ribes*, 4 of which are new, and 52 species of *Grossularia*, 3 of which are new. In addition to the new species, there are many new combinations.

BISBY and BULLER[7] have published a list of the known fungi of Manitoba, which is announced as very incomplete. The list includes 574 species, distributed as follows: Myxomycetes 34, Bacteria 8, Phycomycetes 20, Ascomycetes 62, Fungi Imperfecti 38, Smuts 20, Rusts 80, Polypores 63, Agarics 108, Thelephoraceae 36, other Basidiomycetes 57, Lichens 48.

The third part of THAXTER's monograph of Laboulbeniaceae has just appeared.[8] The two preceding parts have been reviewed in this journal.[9] Since the publication of the second part, the author has published nine preliminary communications appearing in the Proceedings of the American Academy, and including more than 400 species in 14 genera. The major part of the present contribution includes 4 genera of the Dimorphomyceteae; 77 species of *Dimeromyces* parasitic on Coleoptera, Diptera, Orthoptera, and Acarini; and the genus *Chitomyces*, in which 54 species are considered, 9 of which are described as new. This contribution, as the preceding ones, is a model of painstaking and exact work, and further emphasizes the wealth of material in this unique group.

WILDEMAN[10] has investigated the available African material of *Tephrosia*, recording 30 species, 9 of which are described as new.

The Laboratory of Agriculture of San Salvador has published a list of the plants of El Salvador (Central America), under the editorship of STANDLEY and CALDERÓN,[11] with the collaboration of 20 specialists in different groups, and under the auspices of the Smithsonian Institution. Although the list is made up chiefly of angiosperms, there are also a considerable number of fungi, lichens, liverworts, mosses, and ferns.—J. M. C.

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[6] BERGER A., A taxonomic review of currants and gooseberries. N.Y. State Agric. Exp. Sta. Tech. Bull. 109. pp. 118. 1924.

[7] BISBY, G. R., and BULLER, A. H. R., Preliminary list of Manitoba fungi. Trans. Brit. Mycol. Soc. 8:91–109. 1922.

[8] THAXTER R., Contribution toward a monograph of the Laboulbeniaceae. Part III. Mem. Amer. Acad. Sci. 14:315–426. *pls. 12.* 1924.

[9] BOT. GAZ. 23:216. 1897; 47:156. 1909.

[10] WILDEMAN É. DE, Observations sur des espèces Africaines du genre *Tephrosia*. Pers. Bull. Soc. Roy. Bot. Belgique 57: 114–129. 1925.

[11] STANDLEY, P. C., and CALDERÓN S., Lista preliminar de las plantas de El Salvador. pp. 274. 1925.

**Carbon dioxide from roots.**—From a series of experiments on buckwheat, sorghum, soy beans, cow peas, cotton, and velvet beans grown in soil and sand cultures, PARKER[12] presents evidence to show that $CO_2$ production is not related to the feeding power of these plants for calcium, magnesium, phosphorus, or potassium. The measure of feeding power for a given element is taken as the relative abundance or scarcity of the element in the composition of the plant. The studies included: (1) the influence of the crop on the $CO_2$ content of the soil air; (2) the effect of continuous aspiration of the cultures on the feeding power of the plants; (3) the total quantity of $CO_2$ excreted by the roots; (4) the amounts of the different elements absorbed per gram of $CO_2$ given off. Cow peas excreted more $CO_2$ than any of the other plants used, and buckwheat gave off very little $CO_2$, although it had the greatest feeding power. Cotton ranked second in feeding power and sorghum last. Sorghum, soy beans, and cotton were very similar in $CO_2$ production. The removal of $CO_2$ by continuous, rapid aspiration did not influence the composition of the plants. For each gram of $CO_2$ excreted, buckwheat absorbed 41.5 mg. of calcium, soy beans 21.2 mg., cow peas 12.7 mg., and sorghum 5.0 mg.—R. B. DUSTMAN.

**Tertiary flora.**—KUBART[13] has published a contribution to our knowledge of the Tertiary flora. The University of Graz has had a succession of notable paleobotanists, beginning with UNGER, who was followed by ETTINGSHAUSEN, whose laboratory and collections are now in charge of KUBART. The proximity of Tertiary plant deposits has influenced paleobotanical studies at Graz, and a practical side has been given the development of the subject by the present need of more information concerning the lignite beds in Austria.

In the present contribution a careful comparison is made between Tertiary woods and such living woods as can be traced to the Tertiary, as *Sequoia*, *Taxodium*, and *Pseudotsuga*. Not only is the morphological side of the study carried out in detail, but also, by making comparisons with recent swamps in the southern part of the United States and in Mexico, the geological aspect of the origin of Austrian lignite deposits is investigated. Here the author comes to the conclusion that there is no necessity to accept the theory that the Austrian lignite deposits originated only in swamp lands. This may have been the case in many instances, especially at the beginning of the filling out of a land depression, but KUBART believes that the climatic factors of that time favored the preservation of plant material without the formation of extensive swamps in the large *Sequoia* forests of the Tertiary.—A. C. NOÉ.

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[12] PARKER, F. W., Carbon dioxide production of plant roots as a factor in the feeding power of plants. Soil Science 17:229–247. 1924.

[13] KUBART, B., Beiträge zur Tertiarflora der Steiermark nebst Bemerkungen über die Entstehung der Braunkohle. Arbeiten Phytopal. Lab. Univ. Graz I. pp. 62. *pls. 2.* 1924.

# THE

# BOTANICAL GAZETTE

## October 1925

### BIOCHEMISTRY OF PLANT DISEASES

#### V. RELATION BETWEEN SUSCEPTIBILITY TO BROWN ROT IN PLUMS AND PHYSICAL AND CHEMICAL PROPERTIES[1]

J. J. WILLAMAN, N. C. PERVIER, AND H. O. TRIEBOLD

(WITH TEN FIGURES)

An attempt is being made in these investigations to establish the fundamental explanation of differences in susceptibility to brown rot (*Sclerotinia cinerea*) in plum varieties. In previous articles in this series (13, 14) it was reported that, although resistant and susceptible plum varieties differed in respect to density of juice, hydrogen ion concentration, titre, oxalic acid, ash, nitrogen, CaO, and ether extract content, the differences were hardly of sufficient magnitude to be related to the resistance properties. The crude fiber content, however, was conspicuously higher in the two resistant than in the two susceptible varieties. This suggested a physical difference in the texture of the fruit as the basis of the resistance properties. This hypothesis was worked on during the seasons of 1922 and 1923, therefore, and the results are reported in the following paper.

### Previous work

Mechanical resistance on the part of a plant to the entrance of a fungus has been recognized by pathologists for some time as an

[1] Published with the approval of the Director, as Paper no. 561, Minnesota Agricultural Experiment Station.

important means of protection against disease. In 1892 COBB (1) found that certain wheats resistant to rust had thicker leaves, and these leaves had a thicker cuticle, more sclerenchymatous tissue, and greater tensile strength than wheats susceptible to the rust. Later, MELHUS, DURRELL, and KIRBY (9) noted that only the young leaves of the barberry were infected with rust, because the cuticle and epidermis of the older leaves were too thick for the entrance of the rust hyphae. MELANDER (8) actually measured the force required to puncture the young and old leaves of various species of barberry, and found a close relation between resistance and toughness of the leaves.

In 1915 VALLEAU (11) found that those varieties of plums having a thick and tough skin were most resistant to brown rot. He tabulated observations on skin properties and resistance of many different varieties. He also believed that firmness of the flesh, especially at ripeness, played a part in resistance. He made no direct measurements, however, of the force required to penetrate either the skin or the flesh of plums.

So far as the writers are aware, MORRIS of the Washington Agricultural Experiment Station was the first to use a mechanical pressure test on fruits. LEWIS, MURNEEK, and CATE (7) describe an apparatus, the idea for which they obtained from MORRIS. The latter used it to follow the changes in apples during storage, and the former the changes in pears during ripening.

The most accurate instrument for mechanical puncture tests is a modified Jolly balance, the delicate spring and vernier scales of which can readily be adapted for measuring the force necessary for various needles to puncture given tissues. Such an instrument was used by HAWKINS and HARVEY (3), HAWKINS and SANDO (4), MELANDER (8), and by the present writers in the work here reported. HAWKINS and HARVEY found that the reason McCORMICK potato tubers are more resistant to attack by *Pythium debaryanum* than are those of Green Mountain and Bliss Triumph is that the tissue is firmer, and resists in a mechanical way penetration by the fungus. Associated with the firmer texture is a markedly greater crude fiber content. After careful consideration of all observable evidence, these writers concluded that mechanical puncture is the only means this

fungus has of penetrating cell walls; and the mechanical measure of the hardness of the tissues thus becomes of real value. Furthermore, the osmotic pressure of the fungus hyphae was greater than that required to puncture the susceptible tubers, but less than that to puncture the resistant ones.

HAWKINS and SANDO were interested in explaining why small fruits kept in cool storage were less liable to spoilage than those kept at higher temperatures. They found that the average pressure required to puncture cool fruit was greater than that required for warm fruit. This held for strawberries, black raspberries, red raspberries, blackberries, and cherries. Thus the greater toughness of the skin is probably one of the factors in the better keeping of these fruits at lower temperatures.

Since previous work with plums by the present writers had shown that the resistant varieties were higher in crude fiber, it was decided to make a more extensive study of this factor, as well as of the mechanical properties of plum fruits. Eleven varieties of plums were used, covering the whole range of relative susceptibility to brown rot. Crude fiber, pentosan, dry matter, toughness of the skin, and firmness of the flesh were the factors measured.

## Methods and apparatus

Crude fiber was determined by the Kennedy modification of the Sweeney method (6). Pentosans were determined by the method of PERVIER and GORTNER (10). Dry matter was determined by drying in an oven at 100° C. at atmospheric pressure. For the purpose at hand it was considered unnecessary to use any more careful method.

The toughness of the skin was measured by the apparatus shown in fig. 1. After a number of preliminary tests with glass and steel needles, it was decided to use the phonograph needles called "Sonora soft-tone," because of their uniform size, rounded point, and ease of accurate duplication. The needle was sealed into the bottom end of a 10 cc. pipette, cut off within an inch of the bulb. The pipette was filled with mercury until its weight was 40 gm., which was in excess of that required to puncture the toughest plum skin. A wire loop was then sealed into the upper end of the pipette, and the whole suspended from the spring of the balance. To make a determination,
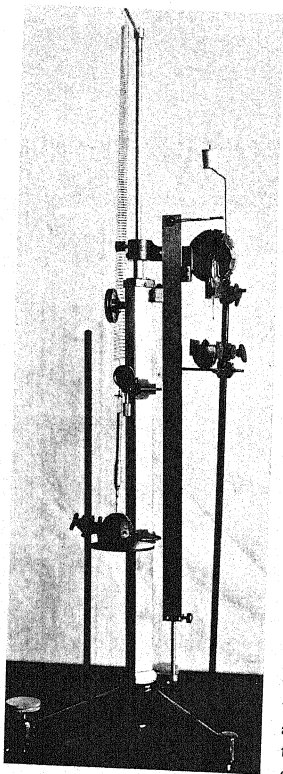
Fig. 1.—Jolly balance adapted for measuring force required to puncture skin and penetrate flesh of plum fruits.

the needle was suspended free on the spring, and the latter lowered until the point of the needle just rested on the surface of the plum, which was held in position on the stand by a clamp. The scale reading was then recorded. The weight of the needle was gradually placed on the plum by releasing the tension of the spring by means of the milled head. At the moment when the needle was observed to break through the skin, the lowering of the spring was stopped and the scale read again. The difference between the two readings indicated the relative amount of force required to press the needle through the skin of the fruit. This force has been recorded directly in centimeters for the sake of simplicity. By the use of the factor 0.9, the readings can be converted into grams, although no useful purpose would be served. Five or six plums of each lot were used, and each plum was tested three times at various points, avoiding the suture. Thus the recorded values are the average of fifteen or eighteen readings. Very concordant values were almost always obtained.

The penetration of the tissue was measured by a harpoon device on the other scale of the Jolly balance. The harpoon consisted of a piece of glass rod, with a no. 10 steel sewing needle fused in one end and a wire hook in the other. The needle was broken off squarely just above the taper, and the broken surface ground smooth, with rounded edges. The needle weighed 3.7067 gm. It was suspended by a thread in front of a small mirror fastened to the scale of the instrument. A few trials showed the approximate height from which the harpoon should be dropped into the plum; this height was marked on the mirror by two horizontal scratches, 18.7 mm. apart. To take a reading, the top point of the harpoon was sighted against the lower mark on the mirror; the plum to be tested was fixed in position so that the surface to be speared was just touching the harpoon; the scale reading was taken; the harpoon was raised until its top was sighted against the upper mark on the mirror; the thread suspending the harpoon was burned; the scale and mirror were lowered until the top of the harpoon again was sighted against the lower mark; the reading was taken, and the dif-ference between the two readings represented the distance the harpoon had penetrated the tissue. A penetration reading as recorded here can thus be defined as the depth in centimeters that a harpoon weighing 3.7067 gm., pointed with a steel needle as described, and falling from a



Fig. 2.—Sketch showing method of preparing plum for harpoon test, and position of harpoon when entering tis-sue.

height of 18.7 mm., will penetrate the freshly cut surface of the flesh of the fruit. The harpoon fell through a short collar of glass tubing, which prevented its falling to one side. The plum to be tested was prepared as shown in fig. 2, the cutting being done with a sectioning razor, and every care being taken not to bruise the tissue. Each plum was usually tested twice on each side of the pit, and five plums in each lot were used. Thus the recorded readings are the averages of from fifteen to twenty determinations.

### Materials

Eleven varieties of plums were selected, grown at the University of Minnesota Fruit Breeding Farm at Excelsior,[2] and representing all degrees of susceptibility to brown rot. The values for the relative resistance assigned by VALLEAU were used. The plums were picked at six stages of maturity: the first stage was when the fruit was wholly green, but just showing signs of incipient yellowing; the fifth was when the plums were ripe for picking; the sixth when they were "dead" ripe; and the second, third, and fourth stages were judged arbitrarily by color. In 1922, several samples were unavoidably missed, but in 1923 a complete set was obtained.

### Experimental results

The data obtained in 1922 are displayed in table I, and those in 1923 in table II. The varieties are listed in decreasing order of resistance, the plus signs indicating VALLEAU's ratings. In order to show the comparative values of the figures graphically, charts were constructed (figs. 3–10). The titles indicate the two methods of grouping the data, so that varieties may be compared at each stage of ripeness, and so that the progress of ripeness within each variety may be followed.

In scanning the charts, it should be remembered that the aim is to show the relation between varieties and their resistance to brown rot. For example, it was previously hypothecated, on the basis of work on potatoes and wheat, that in plums also the structural elements, the toughness of the skin, and the firmness of the tissue would be greatest in the resistant and least in the susceptible varieties. Since the varieties are arranged in order of decreasing resistance, the heights of the columns in the chart should tend to decrease from left to right, except in the case of the penetration data, where the softer tissues give the greater values.

In figs. 3 and 7 the percentage of dry matter bears no apparent relation to resistance properties. The crude fiber, however, has an unmistakable tendency to be higher in the resistant varieties. This is more marked in the later stages of ripeness. It is significant that

these stages are the critical ones for brown rot, since infection seldom takes place in any variety during the very green stages (2). The crude fiber of each variety also decreases as ripeness proceeds (fig. 8), and this is more marked in the susceptible varieties. An exception to this tendency is noticed in many of the "dead" ripe samples (stage VI), where the fiber suddenly increases. This is probably due to the loss of sugars by combustion, and the consequent relative increase of the inert crude fiber.

HAWKINS and HARVEY estimated the pentosan content of potato tubers, to determine whether it as well as the crude fiber contributed to the resistance properties of the tubers. They could find no such relation, however, in the three varieties they tested. The 1922 lots of plums were analyzed for pentosans, with the results shown in fig. 4. There is some evidence of more pentosans in the resistant varieties, and of less in the riper stages; but these tendencies are not so marked as in the case of the crude fiber. Since both of these substances contribute to the structural elements of plant tissues, justification was felt for combining the two values, especially since a test showed that the crude fiber contained no appreciable amount of pentosan. This was done in fig. 5. It is obvious that the varietal differences are enhanced, indicating still more convincingly that the cell wall materials of these fruits play an important part in their resistance to brown rot.

The toughness of the skin of all varieties decreases considerably in the last three stages of ripeness, as the puncture values in figs. 6, 9, and 10 indicate. Furthermore, it decreases more in the susceptible than in the resistant varieties. The differences are marked and very consistent. Also, as would be expected, the tissues become softer as the fruit ripens, and this is more marked in the susceptible varieties. It will be noted that the limiting value of penetration is 3.0 cm. This value was arbitrarily assigned to a sample when the harpoon buried itself to the "hilt," or when the point protruded through the plum, so that perhaps some of these values should be somewhat greater than 3.0 cm. The plums of two resistant varieties, Burbank×Wolf 15 and 9 (fig. 9), in stage VI became as soft as the susceptible, indicating further why differential resistance among varieties tends to disappear as ripening becomes far advanced.

TABLE I

ANALYSES OF PLUM VARIETIES, SEASON OF 1922, LISTED IN ORDER OF DECREASING RESISTANCE TO BROWN ROT

| LABORATORY NO. | DATE PICKED | STAGE OF RIPENESS | PUNCTURE (CM.) | PENETRATION (MM.) | DRY MATTER (PER CENT) | CRUDE FIBER (PER CENT) | | PENTOSANS (PER CENT) | | PENTOSANS+CRUDE FIBER (PER CENT) | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Wet basis | Dry basis | Wet basis | Dry basis | Wet basis | Dry basis |
| | | | | | Burbank×Wolf 15 (15) +* | | | | | | |
| 16 | August 8 | 1 | 21.0 | 0.30 | 11.68 | 0.97 | 8.30 | 0.67 | 5.74 | 1.64 | 14.04 |
| 31 | August 20 | 3 | 23.8 | 0.50 | 8.80 | 1.05 | 11.93 | 0.50 | 5.68 | 1.55 | 17.61 |
| 32 | August 20 | 4 | 22.3 | 0.54 | 10.71 | 0.91 | 8.50 | 0.50 | 4.67 | 1.41 | 13.07 |
| 41 | September 4 | 6 | 17.4 | 1.45 | 8.76 | 0.99 | 11.30 | 0.47 | 5.37 | 1.46 | 16.67 |
| | | | | | Abundance×Wolf 18 (18) +* | | | | | | |
| 6 | August 2 | 2 | 21.6 | 0.22 | 11.11 | 1.12 | 10.08 | 0.75 | 6.75 | 1.87 | 16.83 |
| 30 | August 16 | 3 | 22.5 | 0.40 | 13.00 | 1.01 | 7.77 | 0.73 | 5.62 | 1.74 | 13.39 |
| 36 | August 28 | 5 | 22.0 | 0.78 | 14.90 | 1.03 | 6.91 | 0.60 | 4.03 | 1.63 | 10.94 |
| | | | | | Burbank×Wolf 9 (9) ++* | | | | | | |
| 2 | July 18 | 1 | .... | 0.23 | 10.52 | 0.81 | 7.70 | 0.67 | 6.36 | 1.48 | 14.06 |
| 5 | August 2 | 2 | 21.6 | 0.29 | 12.17 | 0.71 | 5.83 | 0.52 | 4.27 | 1.23 | 10.10 |
| 27 | August 16 | 3 | 25.2 | 0.51 | 12.18 | 0.68 | 5.58 | 0.43 | 3.53 | 1.11 | 9.11 |
| 37 | August 28 | 5 | 20.8 | 1.00 | 12.14 | 0.59 | 4.86 | 0.46 | 3.79 | 1.05 | 8.65 |
| 38 | August 28 | 5 | 18.7 | 1.14 | 12.91 | 0.54 | 4.18 | | | | |
| 42 | September 4 | 6 | 18.6 | 1.25 | | | | | | | |
| | | | | | Burbank×Wolf 12 (12) ++* | | | | | | |
| 14 | August 4 | 2 | 26.3 | 0.34 | 10.59 | 0.81 | 7.65 | 0.54 | 5.10 | 1.35 | 12.75 |
| 19 | August 10 | 3 | 20.4 | 0.59 | 10.79 | 0.67 | 6.21 | 0.55 | 5.10 | 1.22 | 11.31 |
| 29 | August 16 | 4 | 16.8 | 0.92 | 11.77 | 0.68 | 5.78 | 0.46 | 3.91 | 1.14 | 9.69 |

| No. | Date | Sp. | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Burbank×Wolf 21 (21) +++*** | | | | | | | | | | | |
| 3..... | July 28 | 1 | 25.3 | 0.41 | 11.24 | 0.68 | 6.05 | 0.57 | 4.93 | 1.51 | 13.06 |
| 12..... | August 4 | 2 | 26.8 | 0.53 | 11.56 | 0.94 | 8.13 | | 3.60 | 1.39 | 10.22 |
| 17..... | August 8 | 3 | 23.6 | 0.60 | 13.60 | 0.90 | 6.62 | 0.49 | 3.76 | 1.55 | 12.96 |
| 20..... | August 10 | 4 | 25.4 | 0.74 | 11.96 | 1.10 | 9.20 | 0.45 | 3.68 | 1.21 | 9.89 |
| 24..... | August 14 | 5 | 19.4 | 0.77 | 12.23 | 0.76 | 6.21 | 0.45 | 3.90 | 1.13 | 10.02 |
| 33..... | August 20 | 6 | 13.2 | 1.53 | 11.27 | 0.69 | 6.12 | 0.44 | | | |
| **Burbank×Wolf 16 (16) +++*** | | | | | | | | | | | |
| 15..... | August 8 | 1 | 27.2 | 0.35 | 11.27 | 0.74 | 6.57 | 0.55 | 4.88 | 1.29 | 11.45 |
| **Assiniboine (As) ++++** | | | | | | | | | | | |
| 1..... | July 18 | 1 | 18.9 | 0.27 | 13.27 | 0.71 | 5.35 | 0.61 | 4.66 | 1.32 | 10.95 |
| 7..... | August 2 | 3 | 20.7 | 0.64 | 13.17 | 0.67 | 5.09 | 0.50 | 3.80 | 1.17 | 8.80 |
| 13..... | August 4 | 4 | 14.9 | 0.97 | 12.78 | 0.64 | 5.01 | 0.46 | 3.60 | 1.10 | 8.61 |
| 22..... | August 10 | 5 | 11.5 | 3. (ca.) | 13.47 | 0.72 | 5.35 | 0.50 | 3.71 | 1.22 | 9.06 |
| 23..... | August 14 | 6 | | 3. (ca.) | | | | | | | |
| **Abundance×Wolf 30 (30) ++++++*** | | | | | | | | | | | |
| 4..... | July 28 | 1 | 27.1 | 0.28 | 15.28 | 1.04 | 6.81 | 0.54 | 3.72 | 1.51 | 10.41 |
| 28..... | August 16 | 2 | 27.3 | 0.47 | 14.50 | 0.97 | 6.69 | 0.46 | 4.23 | 1.18 | 10.24 |
| 35..... | August 28 | 4 | 22.7 | 0.92 | 11.59 | 0.69 | 5.95 | 0.49 | 4.36 | 0.95 | 8.81 |
| 40..... | August 31 | 5 | 13.2 | 1.24 | 10.79 | 0.48 | 4.45 | 0.47 | | | |
| 43..... | September 4 | 6 | 13.1 | 1.55 | | | | | | | |
| 441..... | September 4 | | 14.8 | 1.76 | | | | | | | |

* += greatest relative resistance; +++= least relative resistance.
† Nos. 34, 41, and 44 were probably unit specimens kept over several days without ice.

TABLE I—*Continued*

| LABORATORY NO | DATE PICKED | STAGE OF RIPENESS | PUNCTURE (CM.) | PENETRATION (MM.) | DRY MATTER (PER CENT) | CRUDE FIBER (PER CENT) | | PENTOSANS (PER CENT) | | PENTOSANS + CRUDE FIBER (PER CENT) | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Wet basis | Dry basis | Wet basis | Dry basis | Wet basis | Dry basis |
| | Emerald (Em) +++* | | | | | | | | | | |
| 21 | August 10 | 1 | 29.6 | 0.29 | 13.68 | 0.89 | 6.51 | 0.65 | 4.75 | 1.54 | 11.26 |
| 34 | August 20 | 2 | 26.4 | 0.50 | 13.62 | 0.74 | 5.43 | 0.62 | 4.55 | 1.36 | 9.98 |
| 30 | August 31 | 3 | 22.7 | 0.98 | 12.44 | 0.59 | 4.74 | 0.56 | 4.50 | 1.15 | 9.24 |
| 45 | September 13 | 5 | 18.2 | 1.49 | 15.14 | 0.59 | 3.90 | 0.51 | 3.37 | 1.10 | 7.27 |
| 46 | September 13 | 6 | 16.3 | 3.(ca.) | 14.33 | 0.59 | 3.49 | 0.51 | 3.56 | 1.01 | 7.05 |
| | Sand cherries (SC) ++++* | | | | | | | | | | |
| 8 | August 2 | 2 | 17.3 | 0.35 | 11.03 | 0.65 | 5.89 | 0.48 | 4.35 | 1.13 | 10.24 |
| 9 | August 2 | 4 | 12.2 | 0.74 | 13.14 | 0.44 | 3.35 | 0.60 | 4.57 | 1.04 | 7.92 |
| 10 | August 2 | 5 | ..... | 3.(ca.) | 13.68 | 0.38 | 2.78 | 0.54 | 3.95 | 0.92 | 6.73 |
| 18 | August 8 | 6 | 4.2 | ..... | 14.42 | 0.35 | 2.43 | 0.44 | 3.05 | 0.79 | 5.48 |
| | Compass (C) ++++* | | | | | | | | | | |
| 11 | August 4 | 3 | 20.4 | 0.66 | 12.68 | 0.65 | 5.13 | 0.64 | 5.05 | 1.29 | 10.18 |
| 25 | August 14 | 5 | 15.1 | 3.(ca.) | 12.98 | 0.60 | 4.62 | 0.35 | 2.70 | 0.95 | 7.32 |
| 26 | August 14 | 6 | 11.5 | 3.(ca.) | 13.93 | 0.41 | 2.94 | 0.47 | 3.37 | 0.88 | 6.31 |

TABLE II

ANALYSES OF PLUM VARIETIES, SEASON OF 1923, LISTED IN ORDER OF DECREASING
RESISTANCE TO BROWN ROT

| LABORATORY NO. | DATE PICKED | STAGE OF RIPENESS | PUNCTURE (CM.) | PENETRA- TION (MM.) | DRY MATTER (PER CENT) | CRUDE FIBER (PER CENT) | |
|---|---|---|---|---|---|---|---|
| | | | | | | Wet basis | Dry basis |
| Burbank×Wolf 15 (15) +* | | | | | | | |
| 19........ | August 9 | 1 | 21.3 | 0.31 | 10.98 | 0.61 | 5.52 |
| 31........ | August 18 | 2 | 17.2 | 0.51 | 10.88 | 0.54 | 4.97 |
| 37........ | August 21 | 3 | 17.1 | 0.66 | 10.36 | 0.48 | 4.63 |
| 46........ | August 25 | 4 | 15.0 | 0.69 | 10.28 | 0.51 | 4.94 |
| 53........ | August 28 | 5 | 12.4 | 1.97 | 10.46 | 0.49 | 4.68 |
| 60........ | September 8 | 6 | 8.4 | 3. (ca.) | 9.89 | 0.84 | 8.50 |
| Abundance×Wolf 18 (18) +* | | | | | | | |
| 15........ | August 7 | 1 | 23.0 | 0.27 | 10.89 | 0.96 | 8.81 |
| 30........ | August 18 | 2 | 17.7 | 0.44 | 13.05 | 0.73 | 5.59 |
| 45........ | August 25 | 3 | 16.2 | 0.54 | 14.20 | 0.50 | 3.52 |
| 55........ | September 1 | 4 | 14.0 | 0.71 | 13.39 | 0.64 | 4.76 |
| 61........ | September 8 | 5 | 12.7 | 1.39 | 14.94 | 0.68 | 4.52 |
| 64........ | September 14 | 6 | 11.4 | 1.26 | 14.51 | 0.66 | 4.57 |
| Burbank×Wolf 9 (9) +* | | | | | | | |
| 25........ | August 9 | 1 | 21.8 | 0.32 | 11.33 | 0.77 | 6.80 |
| 21........ | August 9 | 2 | 21.0 | 0.37 | 12.24 | 0.69 | 5.64 |
| 38........ | August 21 | 3 | 20.3 | 0.55 | 13.02 | 0.49 | 3.73 |
| 44........ | August 25 | 4 | 15.1 | 0.95 | 12.58 | 0.46 | 3.67 |
| 52........ | August 28 | 5 | 11.4 | 1.40 | 12.00 | 0.49 | 4.11 |
| 59........ | September 8 | 6 | 7.7 | 3. (ca.) | 11.25 | 0.52 | 4.60 |
| Burbank×Wolf 12 (12) ++* | | | | | | | |
| 8........ | August 2 | 1 | 26.9 | 0.30 | 9.27 | 0.77 | 8.26 |
| 16........ | August 7 | 2 | 24.0 | 0.35 | 11.39 | 0.71 | 6.28 |
| 17........ | August 7 | 3 | 20.8 | 0.46 | 12.56 | 0.66 | 5.26 |
| 33........ | August 18 | 4 | 11.6 | 0.70 | 13.60 | 0.58 | 4.28 |
| 40........ | August 21 | 5 | 11.2 | 0.97 | 15.69 | 0.48 | 3.06 |
| 42........ | August 25 | 6 | 10.7 | 1.09 | 17.70 | 0.56 | 3.17 |
| Burbank×Wolf 21 (21) ++* | | | | | | | |
| 1........ | July 28 | 1 | 24.5 | 0.45 | 12.01 | 0.63 | 5.28 |
| 9........ | August 4 | 2 | 22.2 | 0.52 | 13.79 | 0.50 | 3.63 |
| 10........ | August 4 | 3 | 22.1 | 0.62 | 14.38 | 0.52 | 3.52 |
| 23........ | August 9 | 4 | 16.1 | 0.60 | 13.78 | 0.44 | 3.18 |
| 27........ | August 14 | 5 | 15.4 | 0.81 | 13.26 | 0.39 | 2.94 |
| 39........ | August 21 | 6 | 10.9 | 1.08 | 13.65 | 0.42 | 3.07 |

* +=greatest relative resistance; ++++=least relative resistance.

TABLE II—*Continued*

| LABORATORY NO. | DATE PICKED | STAGE OF RIPENESS | PUNCTURE (CM.) | PENETRATION (MM.) | DRY MATTER (PER CENT) | CRUDE FIBER (PER CENT) | |
|---|---|---|---|---|---|---|---|
| | | | | | | Wet basis | Dry basis |
| | | Burbank×Wolf 16 (16) ++* | | | | | |
| 14........ | August 7 | 1 | 28.8 | 0.34 | 12.11 | 0.78 | 6.44 |
| 20........ | August 9 | 2 | 24.7 | 0.44 | 12.42 | 0.69 | 5.58 |
| 26........ | August 14 | 3 | 21.1 | 0.48 | 12.35 | 0.60 | 4.84 |
| 32........ | August 18 | 4 | 14.5 | 0.63 | 13.94 | 0.57 | 4.08 |
| 43........ | August 25 | 5 | 12.9 | 1.24 | 14.24 | 0.40 | 2.83 |
| 54........ | September 1 | 6 | 8.6 | 2.20 | 12.08 | 0.49 | 4.07 |
| | | Assiniboïne (As) +++* | | | | | |
| 2........ | July 28 | 1 | 23.5 | 0.31 | 10.59 | 0.86 | 8.12 |
| 7........ | August 2 | 2 | 20.5 | 0.45 | 11.96 | 0.72 | 6.00 |
| 13........ | August 4 | 3 | 18.8 | 0.55 | 12.21 | 0.80 | 6.53 |
| 22........ | August 9 | 4 | 14.0 | 1.35 | 11.46 | 0.46 | 4.02 |
| 28........ | August 14 | 5 | 9.3 | 3.(ca.) | 11.71 | 0.47 | 4.00 |
| 34........ | August 18 | 6 | 7.2 | 3.(ca.) | 10.54 | 0.48 | 4.58 |
| | | Abundance×Wolf 30 (30) ++++* | | | | | |
| 35........ | August 18 | 1 | 20.5 | 0.43 | 14.76 | 0.56 | 3.79 |
| 49........ | August 25 | 2 | 17.4 | 0.75 | 14.59 | 0.55 | 3.73 |
| 50........ | August 25 | 3 | 16.3 | 0.76 | 13.89 | 0.57 | 4.09 |
| 56........ | September 1 | 4 | 11.0 | 1.05 | 14.07 | 0.49 | 3.50 |
| 62........ | September 8 | 5 | 8.9 | 3.(ca.) | 14.40 | 0.46 | 3.22 |
| 65........ | September 14 | 6 | 7.6 | 3.(ca.) | 13.16 | 0.50 | 3.78 |
| | | Emerald (Em) ++++* | | | | | |
| 36........ | August 18 | 1 | 21.9 | 0.45 | 12.04 | 0.62 | 5.13 |
| 47........ | August 25 | 2 | 16.1 | 0.67 | 11.11 | 0.54 | 4.83 |
| 48........ | August 25 | 3 | 14.5 | 0.75 | 12.98 | 0.57 | 4.38 |
| 57........ | September 1 | 4 | 11.3 | 1.22 | 13.05 | 0.58 | 4.43 |
| 63........ | September 8 | 5 | 8.1 | 3.(ca.) | 13.02 | 0.61 | 4.68 |
| 66........ | September 14 | 6 | 7.5 | 3.(ca.) | 12.37 | 0.65 | 5.28 |
| | | Sand cherries (SC) ++++* | | | | | |
| 3........ | July 28 | 1 | 17.6 | 0.37 | 7.35 | 0.75 | 10.12 |
| 4........ | August 2 | 2 | 18.5 | 0.44 | 8.39 | 0.71 | 8.40 |
| 18........ | August 7 | 3 | 15.8 | 0.54 | 7.97 | 0.62 | 7.74 |
| 29........ | August 14 | 4 | 11.4 | 3.(ca.) | 8.72 | 0.38 | 4.34 |
| 41........ | August 21 | 5 | 7.7 | 3.(ca.) | 9.51 | 0.32 | 3.41 |
| 58........ | September 1 | 6 | 4.9 | 3.(ca.) | 9.99 | 0.38 | 3.80 |

TABLE II—*Continued*

| LABORATORY NO. | DATE PICKED | STAGE OF RIPENESS | PUNCTURE (CM.) | PENETRA- TION (MM.) | DRY MATTER (PER CENT) | CRUDE FIBER (PER CENT) | |
|---|---|---|---|---|---|---|---|
| | | | | | | Wet basis | Dry basis |
| | | | Compass (C) ++++* | | | | |
| 5........ | August 2 | 1 | 20.1 | 0.35 | 10.74 | 0.67 | 6.25 |
| 6........ | August 2 | 2 | 15.6 | 0.66 | 10.03 | 0.57 | 5.67 |
| 11........ | August 4 | 3 | 13.1 | 0.83 | 11.23 | 0.43 | 3.80 |
| 12........ | August 4 | 4 | 12.2 | 1.23 | 10.36 | 0.44 | 4.20 |
| 24........ | August 9 | 5 | 10.5 | 3.(ca.) | 9.40 | 0.36 | 3.81 |
| 51........ | August 25 | 6 | 5.2 | 3.(ca.) | 13.53 | 0.35 | 2.58 |

The relation between the skin and the flesh texture of the plums can be shown in a different way graphically by plotting the two values against each other, and connecting the values of a single variety by a line. It is apparent that in general, as ripening proceeds, the flesh becomes softer and the skin tenderer; but that during the last stages the flesh softens far more rapidly than does the skin.

Since we are here obviously dealing with several correlated pairs of factors, it was thought desirable to find the mathematical value of these correlations. Because of the small number of samples involved, it was felt that the calculation of the standard coefficient of correlation was not justified; hence correlation by rank was calculated for the 1923 samples, using the formula given by JACKSON (5): $r = 1 - 6(\Sigma D_k^2)/(n^3 - n)$. The probable error was calculated by the following formula, taken from WHIPPLE (12): $e = 0.706 \times \dfrac{1 - r^2}{\sqrt{n}}$. The values for the factors of the eleven varieties for a given stage of maturity were used in the calculations. The results are given in table III.

Although in a way the group of individuals used in each case may be considered heterogeneous, because it consisted of numerous varieties instead of but one, it was felt that if, in spite of this, coefficients of some magnitude did appear, they would certainly be significant. Furthermore, the signs of the coefficients for a series would perhaps in themselves have a meaning. Because $n$ is only 11 in all cases, the probable error is large; and it will be considered that the coefficients are significant only when they have a value of 0.48 or

FIG. 3.—Dry matter and crude fiber of 1922 samples of plums, grouped for comparison of varieties at each stage of ripeness; Roman numerals indicate stages of ripeness, numbers and letters indicate names of varieties (latter from left to right in order of increasing susceptibility to brown rot).

FIG. 4.—Pentosan content of 1922 samples of plums, grouped for comparison of varieties at each stage of ripeness; Roman numerals indicate stages of ripeness, numbers and letters indicate names of varieties (latter from left to right in order of increasing susceptibility to brown rot).

FIG. 5.—Pentosan and crude fiber content added together, and grouped for comparison of varieties at each stage of ripeness; Roman numerals indicate stages of ripeness, numbers and letters indicate names of varieties (latter from left to right in order of increasing susceptibility to brown rot).

FIG. 6.—Puncture and penetration data of 1922 samples of plums, grouped for comparisons of varieties at each stage of ripeness; Roman numerals indicate stages of ripeness, numbers and letters indicate names of varieties (latter from left to right in order of increasing susceptibility to brown rot).
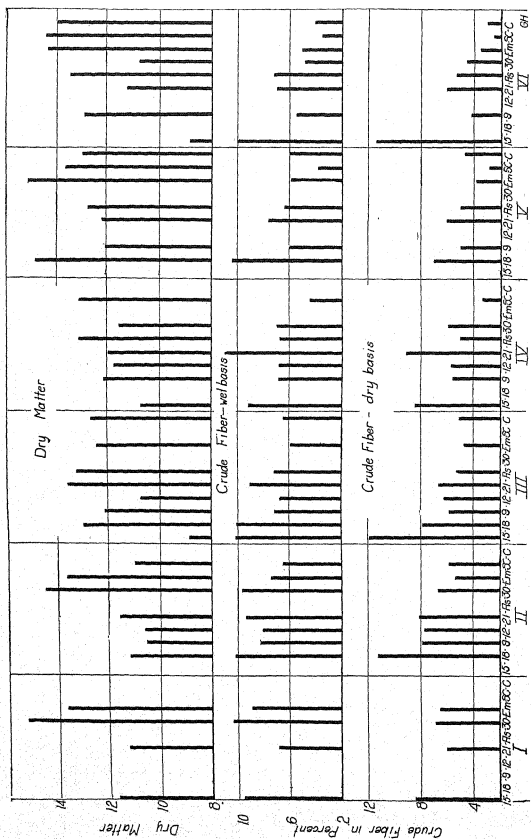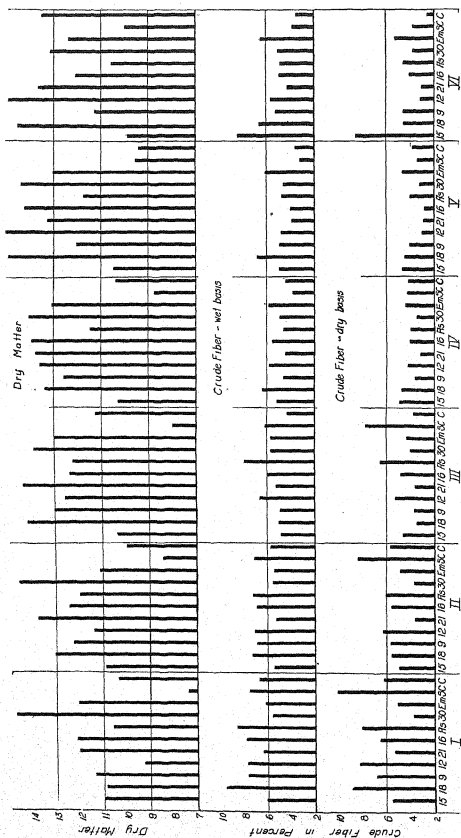
FIG. 7.—Dry matter and crude fiber of 1923 samples of plums, grouped for comparison of varieties at each stage of ripeness; Roman numerals indicate stages of ripeness, numbers and letters indicate names of varieties (latter from left to right in order of increasing susceptibility to brown rot).

FIG. 8.—Dry matter and crude fiber of 1923 samples of plums, grouped to show progress of ripening in each variety; samples placed according to dates of picking as well as according to stages of ripeness; Roman numerals indicate stages of ripeness, numbers and letters indicate names of varieties (latter from left to right in order of increasing susceptibility to brown rot).
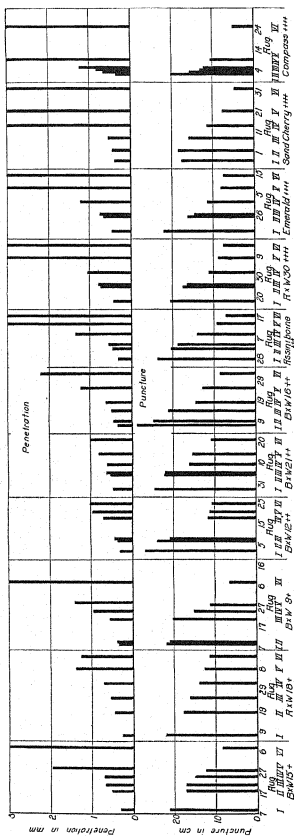
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FIG. 9.—Puncture and penetration data for 1923 samples of plums, grouped for comparison of varieties at each stage of ripeness; Roman numerals indicate stages of ripeness, numbers and letters indicate names of varieties (latter from left to right in order of increasing susceptibility to brown rot).

FIG. 10.—Puncture and penetration data for 1923 samples of plums, grouped to show progress of ripening in each variety; samples arranged by dates as well as by stages of ripeness; Roman numerals indicate stages of ripeness, numbers and letters indicate names of varieties (latter from left to right in order of increasing susceptibility to brown rot).

TABLE III

CORRELATIONS BY RANK AMONG VARIOUS FACTORS IN ELEVEN VARIETIES OF PLUMS; DATA OF 1923

| STAGE | PUNCTURE AND PENETRATION | PENETRATION AND DRY MATTER | PENETRATION AND CRUDE FIBER (WET BASIS) | PUNCTURE AND CRUDE FIBER (WET BASIS) | DRY MATTER AND CRUDE FIBER (WET BASIS) | PUNCTURE AND DRY MATTER |
|---|---|---|---|---|---|---|
| 1 | $-0.263 \pm 0.193$ | $+0.486 \pm 0.163$ | $-0.638 \pm 0.126$ | $+0.480 \pm 0.162$ | $-0.411 \pm 0.177$ | $+0.181 \pm 0.206$ |
| 2 | $-0.664 \pm 0.119$ | $+0.109 \pm 0.209$ | $-0.630 \pm 0.128$ | $+0.289 \pm 0.195$ | $-0.057 \pm 0.212$ | $+0.400 \pm 0.179$ |
| 3 | $-0.504 \pm 0.155$ | $+0.037 \pm 0.212$ | $-0.556 \pm 0.147$ | $+0.289 \pm 0.195$ | $-0.116 \pm 0.210$ | $+0.340 \pm 0.187$ |
| 4 | $-0.611 \pm 0.134$ | $-0.403 \pm 0.167$ | $-0.408 \pm 0.166$ | $-0.147 \pm 0.205$ | $-0.421 \pm 0.177$ | $-0.032 \pm 0.213$ |
| 5 | $-0.762 \pm 0.089$ | $-0.477 \pm 0.162$ | $+0.075 \pm 0.212$ | $+0.142 \pm 0.209$ | $+0.410 \pm 0.177$ | $+0.340 \pm 0.187$ |
| 6 | $-0.591 \pm 0.141$ | $-0.481 \pm 0.162$ | $+0.064 \pm 0.213$ | $+0.518 \pm 0.156$ | $+0.027 \pm 0.213$ | $+0.545 \pm 0.149$ |

greater, which is about three times the probable error. It is with these considerations in mind that the values in table III should be scanned. The puncture by penetration coefficients bear negative signs in all stages of ripeness, and they are of significant magnitude in all but the first stage. This means, of course, that a positive relation exists between toughness of skin and firmness of tissue. In table III it will be seen in the green samples (stage I) that softer tissue is correlated with higher dry matter; that the value of this coefficient decreases during the next two stages, and then reverses in sign, so that in the last three stages the firmer tissues are correlated with higher dry matter. The coefficients for penetration and crude fiber are all negative, but they decrease in value regularly from the first to the last stage. This indicates that up to the riper stages a firm flesh is related to a high crude fiber, but that this relation disappears at full ripeness.

A tough skin and high crude fiber occur together in the greenest samples and in the "dead" ripe samples; in the intermediate stages these relations do not hold. The writers interpret this as follows. In the very green and in the very ripe fruits, the skin itself contributes largely to the total crude fiber, due in the first case to the relatively large proportion of skin in the half grown fruit, and in the second case to the disintegration of the fiber of the

flesh during over-ripening. The coefficients for dry matter and crude fiber are of significant magnitude only in stage IV; in the other stages they vary erratically. No explanation for this is offered. In the last column of table III it appears that in the over-ripe samples, soft tissue is related to high dry matter, but that these two factors are not significantly related at any other stage. No explanation of this is offered.

## Summary and conclusions

1. The attempt has been made to relate the resistance and susceptibility of plum varieties to brown rot to their chemical and mechanical characteristics. To this end eleven varieties were analyzed at six stages of ripeness during the seasons of 1922 and 1923. Crude fiber, pentosans, and dry matter analyses were made, and the toughness of the skin and the firmness of the flesh were measured.

2. It has been found in general that the resistant varieties have a higher crude fiber content than the susceptible, and that this holds more in the ripe than in the unripe stages of maturity.

3. The pentosan content shows somewhat the same relations to susceptibility as does the crude fiber, but to a less degree. When the two factors are added, in order to get a greater index of the structural elements of the fruit tissue, the relation to susceptibility is more marked than when either is considered alone.

4. Although the toughness of the skin decreases in all varieties as ripeness proceeds, the change is more marked in the susceptible varieties. This is quite in accord with the resistance of plums to brown rot, since varietal differences are not apparent in the greener stages.

5. The firmness of the flesh of plums parallels to a striking degree the toughness of the skin in all stages of maturity. Unquestionably both factors are of importance in conferring resistance on a variety.

6. When the plums become ripe, and especially when over-ripe, these relations tend to disappear; and at these stages practically all varieties become susceptible to brown rot.

7. Coefficients of correlation by rank were calculated for all pairs of factors, and for all stages of maturity, for the 1923 samples. The values obtained verify the generalizations consistently.

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# PHYSIOLOGICAL STUDY OF TWO VARIETIES
## OF IPOMOEA BATATAS

### George R. Johnstone

#### (WITH FOUR FIGURES)

### Introduction

As an agricultural crop, the sweet potato is second in importance to the Irish potato in the United States. Its production has been increasing in recent years, however, which attaches an increasing importance to the problems of conservation and prevention of losses in storage and in marketing. The average acreage for the years 1910–1914 was 611,000, while in 1920 there were 1,085,000 acres devoted to this crop (33). Losses due to disease have been estimated at $58,000,000 for the year 1919. Although improved methods of curing and storing have increased its market value, and have decreased the losses due to decay from 36.23 per cent in 1919 to 21.45 per cent in 1922 (24), it is evident that there is great need of fundamental physiological research on cured and uncured sweet potatoes, and on the problems of internal breakdown, discoloration, etc., in different types, in order that more adequate conservation measures may be employed.

The aim of this investigation, therefore, has been to obtain more information regarding the physiology of the sweet potato, particularly with respect to respiration, certain enzymatic activities, and water relations. Hasselbring and Hawkins (10) studied respiration in the Jersey Big Stem, a dry, firm fleshed variety. Their experiments were carried out at a temperature of 30° C. They found that freshly dug samples increased their rate of respiration for a few days and then the rate decreased. If, however, they were left in the laboratory at 20° C. for 18 days, and then placed in the respiratory apparatus, they reached a maximum rate more quickly. Similar experiments with cured sweet potatoes (that is, those which had been

subjected to a drying process in a well ventilated room at 30° C. for 10 days), after having been stored at 6°–7° C. for 32 days, respired at a much greater rate when placed at the higher temperature for making the determination. This rate gradually decreased to about one half of the maximum on the tenth day, while those which were cured and stored at 12°–15° showed only a slight increase followed by a slight decrease, under the same conditions.

Parallel with these experiments on respiration, analyses were made to determine whether there was any correlation between seasonal changes in sugar content and respiratory activity. Conclusions were negative as to any such general correlations. The respiratory changes effected by splitting the sweet potatoes lengthwise were also determined. Before the roots were split, they respired at the rate of 29.8–42.7 mg. per kilogram hour. After they were split, the rate was 60 mg., which finally decreased to 52.5 mg. on the seventh day., The earlier explanation for the increased respiration of wounded tissues was that wounding acts as a stimulus to increase oxidation. The recent work of MAGNESS (17), however, shows that the removal of the epidermal or cortical structures of apples and potatoes is followed by an increase in the oxygen content of the intercellular atmosphere. Since an increase in oxygen pressure is known to hasten respiration of plant tissues, it is probable that the increased respiration occurring in wounded tissues is due largely to facilitation of gaseous exchange in the cut areas. The increased respiration in split sweet potatoes may then be due to an increase in the available oxygen, rather than to other kinds of wound stimulation.

Among other things which have a bearing on the curing and storing of sweet potatoes, is the empirical method suggested by THOMPSON (31) for telling when the former process is complete. He referred to the appearance of sprouts as a criterion. MANNS (19) has made extensive storage studies. He attributes the loss in weight during storage to the loss in moisture, and recommends a temperature of 55° F., with a relative humidity of 60 per cent for storage. Humidity above 80 per cent is very favorable for storage rots. PRICE (25) has found that losses due to harvesting after frost amounted to 67.7 per cent of Porto Rico variety in storage, and 80 per cent of Triumph, as compared with harvesting before frost, when

the losses were only 0.8–1.25 per cent of the first variety and 0.4–5 per cent of the second.

With respect to work on enzymes, GORE (7) has determined the diastatic activity for a few varieties of sweet potato, and has worked out a method whereby the contained diastase can be utilized in the preparation of sweet potato sirup. MAGOON and CULPEPPER (18) refer to an oxidase as accounting for discoloration of sweet potatoes before cooking, but show no data regarding it. SHERMAN and BALDWIN (28) have shown that the optimum hydrogen ion concentration for the activity of three typical amylases varies considerably with reference to the source of the enzyme. The experimental work which follows deals with a number of physiological problems connected with harvesting, storing, and keeping qualities of sweet potatoes, especially with regard to the respiratory and enzymic activities under various conditions, and incidentally to determine whether there is a physiological grouping of varieties comparable with that obtained by STEENBOCK and SELL (29), which was based on the fat-soluble vitamine content.

## Materials and methods

The sweet potatoes used for the following experiments were obtained from W. A. GARDNER of the Agricultural Experiment Station, Auburn, Alabama, and from J. C. C. PRICE of the Mississippi A. and M. College, Mississippi, to whom my thanks are gratefully accorded. Since most of the material was obtained from the former station, the origin will not be indicated below except when it is from the other source. The approximate 900 miles transit has added to the variable uncontrollable environmental factors which must be taken into consideration.

RESPIRATION.—An apparatus similar to that of MAGNESS (16), but adapted for water bath temperature control was used to determine the amount of oxygen taken up and carbon dioxide released during respiration at 25° C. Fig. 1 is a diagram of the apparatus. The apparatus was maintained at 25° C. in a Freas water bath submerged to the point $W$. One hour was allowed for the adjustment of the apparatus to that of the bath, then the separatory funnel was closed. Any significant errors due to vapor pressure, temperature,

etc., were corrected by means of a control apparatus, which was connected with a water manometer for reading very slight changes in volume. The amount of carbon dioxide liberated by the respiring material was determined by titrating an aliquot part of the potassium hydroxide ($K$), using the double indicator method (6). At the end of the experiment the contained gases were again adjusted to
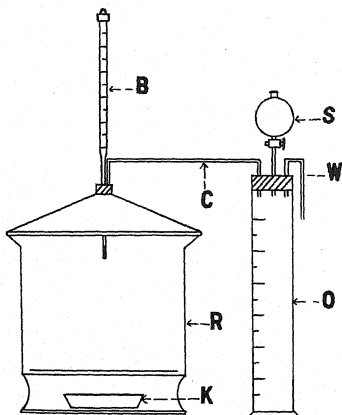


Fig. 1.—Respiratory apparatus adapted for water bath temperature control: $R$, respiratory chamber; $K$, 100 cc. potassium hydroxide; $B$, Beckmann thermometer; $C$, capillary tube; $S$, separatory funnel; $W$, water intake tube; $O$, oxygen cylinder.

atmospheric pressure at the water level ($W$), by introducing a little water through the separatory funnel. The volume of water in the oxygen cylinder was taken as equal to the amount of oxygen used by the respiring material. This volume was reduced to standard conditions.

MEASUREMENT OF INTERCELLULAR GASES.—The apparatus designed by MAGNESS (17) was used for taking samples of the intercellular gases, which were transferred for analyses to the Bonnier-Mangin apparatus as described by AUBERT (2).

EFFECT OF INJURY ON RESPIRATION.—Two sets of material from the same variety, Porto Rico, were tested for normal respiration, and then a plug was cut out of each potato by means of a cork borer 16 mm. in diameter. The plugs of one set were replaced and sealed, while they were left out of the other set, but included in the respiratory chamber. An equal normal area of the latter was sealed to correspond to that of the former, and then the respiratory activity of each set was determined in the usual manner.

MOISTURE DETERMINATIONS.—The stem half was used in each case for moisture determination. It was ground on a potato grater to a fine pulp, and 4–6 gm. samples of it were placed on tared watch glasses and weighed. The material was then covered with 95 per cent alcohol, and dried to constant weight at 60° C. in a vacuum oven at reduced pressure.

CURING TEMPERATURE AND REGENERATION.—Sweet potatoes kept at 30° C. for 10 days in the curing house frequently develop shoots 2–4 cm. long or longer. The question has occurred as to whether the development of these shoots was initiated by the higher temperature, or by a less likely cause, namely, the loss of water which occurs during the process. In order to obtain some information bearing on this question, two similar lots were selected, and placed in desiccators at 27–30° C. for a period of 10 days. The bottom of one desiccator was covered with water, while a dish of $CaCl_2$ was placed in the other. Air was drawn through both desiccators by means of a suction pump. The air drawn through the former was first bubbled through concentrated sulphuric acid and then through water, so that a nearly saturated atmosphere was maintained practically free from ammonia and other obnoxious gases. In the other desiccator, a dry atmosphere was maintained by drawing air into it through a calcium chloride tube and then through concentrated sulphuric acid.

HYDROGEN-ION CONCENTRATION.—By means of the hydrogen electrode, the hydrogen-ion concentration of the expressed juices of each variety was determined. After equilibrium in the solutions being tested was established, readings in millivolts on the type $K$ potentiometer were made, and later converted into $P_H$ values, correcting for temperature according to the method described by MICHAELIS (20).

CATALASE.—The apparatus used and the method employed for the determination of catalase activity were essentially those of APPLEMAN (1). Because of insufficient material, it was impossible to take the juice from portions of several sweet potatoes to test as an average, so one was selected as representative of the lot. The first inch of the stem end was discarded; then by means of a 10 mm. cork borer, a central cylinder from the next inch was removed and placed into a glass stoppered weighing bottle. Each test consisted of four or five determinations, by weighing out different quantities so as to include the mass of material which would give the optimum or very nearly the optimum activity with 5 cc. of standardized hydrogen peroxide, neutralized with calcium carbonate. Each weighed sample of material was triturated in a mortar with a small amount of clean sand, and an equal amount of calcium carbonate. All determinations were made at 25° C., and the volume of oxygen liberated was reduced to standard conditions.

AMYLASE.—WOHLGEMUTH's (9) method was used to demonstrate the amylase. One hundred gm. of the pulp were extracted for one hour at 25° C. with 200 cc. of distilled water, and 1 cc. of toluene was added as a preservative. The extract was filtered through a creased filter paper, and its amylolytic activity was determined by use of a series of 10 test-tubes, each containing 5 cc. of a 1 per cent solution of Merck's soluble starch, maintained at 0° C. in ice water until the extract to be tested was added. The amount of diastase extract was varied, so that the series ranged from 0.2 cc. to 2.0 cc. The preparations were then incubated at 38° C. for 30 minutes, and again returned to the ice water to check hydrolysis. Of those tubes which showed no blue color with N/10 iodine solution, the one that contained the most extract was selected as the standard, and the activity was calculated for 1 cc. of the extract for comparative results expressed as $D38° 30'$. $D$ equals the number of cubic centimeters of 1 per cent starch solution hydrolyzed at 38° C. during a period of 30 minutes (table IX).

LACCASE AND PEROXIDASE.—The following reagents were used to demonstrate the presence of laccase: 0.5 per cent alcoholic solution of gum guaiacum (13), and a 1 per cent aqueous solution of tetramethylparaphenylendiamine hydrochloride (32). These reagents

were used separately by applying them directly to the tissues, and noting the time required for definite color changes to take place. The organic peroxide which was demonstrated to be present by means of starch, KI solution, and acetic acid, was destroyed by heating the juice or tissue to 50° C. for a few minutes. Tests for peroxidase were made by treating the juice or tissue with the same solution of guaiacum previously described in the presence of hydrogen peroxide. Benzidine with hydrogen peroxide seemed to be less satisfactory. The oxidase system, which will be mentioned later, refers to the functioning of the so-called enzymes, laccase and peroxidase, which account for the discoloration of the tissue on exposure to air.

### Experimental data

RESPIRATION AT DIFFERENT DATES OF HARVESTING.—Fortunately no rains occurred in the field where the sweet potatoes were growing until October 16, so that a considerable number of data

TABLE I

RESPIRATION OF UNCURED SWEET POTATOES AT DIFFERENT DATES
OF HARVESTING

| DATE OF HARVEST | DAYS IN TRANSIT | RATE OF RESPIRATION PER KGMHR | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Porto Rico | | | | Triumph | | | |
| | | CO₂ (mg.) | CO₂ (cc.) | O₂ (cc.) | $\frac{CO_2}{O_2}$ | CO₂ (mg.) | CO₂ (cc.) | O₂ (cc.) | $\frac{CO_2}{O_2}$ |
| September 1..... | 6 | 42.63 | 21.71 | 24.61 | 0.882 | 38.80 | 19.76 | 20.71 | 0.954 |
| September 1..... | 7 | 42.99 | 21.89 | 22.28 | 0.982 | 38.13 | 19.42 | 17.55 | 1.10 |
| September 14.... | 9 | 52.92 | 26.95 | 20.59 | 1.300 | 37.40 | 19.04 | ...... | ...... |
| September 14.... | 10 | 57.96 | 29.06 | 27.72 | 1.040 | 39.50 | 20.11 | 15.13 | 1.32 |
| September 27.... | 8 | 56.62 | 28.83 | 27.26 | 1.050 | 37.33 | 19.01 | 14.25 | 1.33 |
| September 27.... | 9 | 73.02 | 37.19 | 35.63 | 1.043 | ...... | ...... | ...... | ...... |
| October 25....... | 5 | 50.17 | 25.55 | 22.51 | 1.145 | 37.94 | 19.32 | 15.54 | 1.24 |
| October 25....... | 9 | 85.17 | 43.38 | 42.60 | 1.018 | 43.17 | 21.98 | 21.63 | 1.01 |

were obtained during favorable weather conditions. Table I shows the rates of respiration for the two varieties at different times of harvesting.

Respiration in the first three sets harvested was determined on two successive days, as indicated in the data of table I. On the

second day in every case there was an appreciable increase. The increase in the rate was more pronounced when the second determination was made after a longer interval, as shown by the data for those harvested October 25. Fig. 2 shows the progress of respiration in storage for the two varieties of uncured sweet potatoes.

Cured sweet potatoes do not reach as high a maximum of respiratory activity as uncured samples under approximately the same conditions of storage, as shown by the curves in fig. 3 compared with those in fig. 2.

The slight increase in respiration shown by the curves on the fourth to seventh days is undoubtedly due to transferring the sweet potatoes to the higher temperature for respiration determination,

TABLE II

RESPIRATION AND TRAUMA AT 25° C., PORTO RICO VARIETY

| TREATMENT | WEIGHT OF SAMPLES (GM.) | | AREA OF INJURED SURFACE IN MM.$^2$ | CO$_2$ RELEASED PER KGMHR (MG.) | | PERCENT-AGE INCREASE |
|---|---|---|---|---|---|---|
| | First | Final | | Before injury | After injury | |
| Plugs removed........... | 632 | 623 | 7385.3 | 64.27 | 126.81 | 97.30 |
| Plugs replaced and sealed.. | 632 | 628 | 7636.4 | 60.06 | 70.36 | 17.15 |

but cured sweet potatoes do not respond as readily to such a change of condition as the uncured.

RESPIRATION AND TRAUMA.—THOMPSON (31) reports that the minimum shrinkage under commercial handling was 7.40 per cent, and maximum 20.40 per cent, with an average of 10 per cent for several lots. The average amount of decay was 0.45 per cent under careful handling and 3 per cent under commercial handling. The data of table II have a bearing on the cause of shrinkage due to commercial handling, but would not account for all of it. One may conclude from this that the part of shrinkage due to respiration, although it may be small, is very much greater when the cortical layer is broken, and that such increase is largely due to facilitating the exchange of gases rather than to direct wound stimulation.

ANALYSIS OF INTERCELLULAR GASES.—Relatively large samples of gas could easily be obtained from cylindrical plugs cut out by
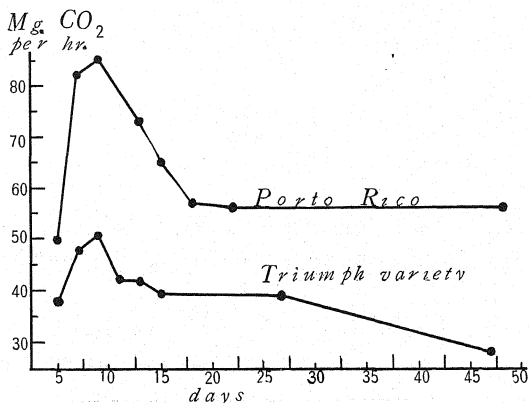
FIG. 2.—Progress of respiratory activity determined at 25° C. at various intervals from date of harvesting for two varieties of sweet potatoes stored in basement at 15°–20° C.
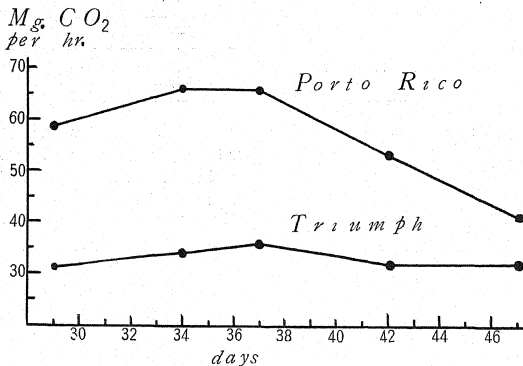


FIG. 3.—Progress of respiration determined at 25° C. for two varieties of cured sweet potatoes in basement storage at 15°–20° C.

means of a 22 mm. cork borer. Gas was drawn out of these plugs by placing them in an apparatus designed by MAGNESS (17). Although a great many determinations were made, only a few typical analyses are given in table III. The larger amount of carbon dioxide in the tissues of Porto Rico variety may be explained as due to the greater rate of respiration which occurs in that variety. Any difference in the porosity of the tissues of the two varieties, or any factor which would facilitate the exchange of gases, would influence the $CO_2/O_2$ ratio.

RESPIRATORY COEFFICIENT.—$Q_{10}$, or the coefficient for each $10°$ rise in temperature, has been worked out for respiration of a number

TABLE III

ANALYSIS OF INTERCELLULAR GASES

| DATE OF HARVEST | DATE OF DETERMINATION | PERCENTAGE | |
| --- | --- | --- | --- |
| | | $CO_2$ | $O_2$ |
| Porto Rico | | | |
| October 25.......... | November 9 | 7.086 | 15.74 |
| | | 7.732 | 14.95 |
| Triumph | | | |
| October 25.......... | November 7 | 6.770 | 15.62 |
| | | 6.780 | 16.13 |

of plants. In raising the temperature of the apparatus and the material each $10°$ a source of error was found, depending on whether the determination was begun immediately, or not until the material had been raised to the desired temperature. If the latter method was used, which was more accurate, the error could not entirely be eliminated, because the material had to be taken out of the respiratory apparatus in order to introduce fresh alkali. This procedure permitted the temperature of the material and the apparatus to fall so as to introduce an error. Both methods were used, and the latter, as expected, gave a steeper curve for the rate of respiration. Both varieties used in these experiments were obtained from the Mississippi Experiment Station. They had been cured and apparently

were in good condition, although the experiments were begun March 26.

RESPIRATION AT HIGHER TEMPERATURE.—Porto Rico variety was used for these determinations. They were cured sweet potatoes from the Mississippi A. and M. College, stored since March 4 in the basement at $15°-20°$ C. In each determination the sweet potatoes were placed in the oven, outside of the respiratory chamber, until their temperature reached that of the oven as indicated by a thermometer inserted into a control sweet potato. When the temperature of its interior became equal to that of the oven, the other sweet potatoes were placed in the respiratory chamber. Thus the period of "preliminary" heating was different for each of the three higher temper-

TABLE IV

TEMPERATURE COEFFICIENT OF RESPIRATION, $Q_{10}$

| TEMPERATURE °C. | CO₂ PER KGMHR (MG.) | $Q_{10}$ | MG. CO₂ PER KGMHR | $Q_{10}$ |
|---|---|---|---|---|
| | Porto Rico | | Triumph | |
| 15................ | 37.35 | ........ | 27.64 | ........ |
| 25................ | 79.22 | 2.121 | 63.31 | 2.29 |
| 35................ | 121.05 | 1.528 | 110.73 | 1.748 |

atures, being one and one-third hours for the experiment conducted at $44°$ C., three hours for that at $50°$, and five hours for the $55°$ series. The curves in fig. 4 indicate the nature of respiration at these temperatures.

The potassium hydroxide which was used to collect the carbon dioxide was changed every two hours for ten hours, titrating it each time to determine the carbon dioxide content.

BLACKMAN (5) has already observed this temperature effect. When a process is determined as to its rapidity by a number of separate factors, the rate of the process is limited by the pace of the slowest factor. He refers to the optimum by some investigators as the highest temperature which can be permanently sustained without depression of function, but more usually a real optimum is held to be characterized by this, that the retardation produced by exposure to superoptimal temperature must not be of the nature of

permanent injury, and that therefore on cooling again to the optimum temperature there must be a return of the function to its highest value. The curve for respiration at 44° C. (fig. 4) shows a slight falling off in the rate.

MOISTURE DETERMINATIONS.—These measurements were made in the usual manner as soon as the material arrived at the laboratory. The first six lots of sweet potatoes had not been cured, while the
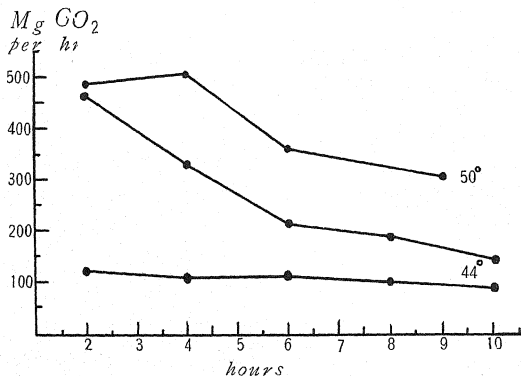


FIG. 4.—Respiration at higher temperatures for cured Porto Rico sweet potatoes; March, 1924.

seventh or last lot was cured. The dates of harvest and dates of moisture determinations are indicated in table V.

It was impossible to take the moisture determinations immediately after curing, because of the delay in transit from Auburn, Alabama. This delay, to a certain extent, would account for the increase in moisture content of Porto Rico variety after curing.

In order to obtain more accurate data regarding the effect of curing on the moisture content, samples of Triumph variety were placed in two desiccators. A moist atmosphere was maintained in one by means of a layer of water, while calcium chloride was placed in the other desiccator to maintain a dry atmosphere. By means of a

suction pump, a continual stream of air was drawn through each desiccator. The air was first drawn through a bottle of sulphuric acid and then through water, to maintain a moist atmosphere in one

TABLE V

MOISTURE DETERMINATIONS AT DIFFERENT DATES OF
HARVESTING

| DATE OF HARVEST | DATE OF DETER-MINATION | PERCENTAGE MOISTURE | |
|---|---|---|---|
| | | Porto Rico | Triumph |
| August 2............ | August 9 | 76.11 | 73.07 |
| | | 76.29 | 73.52 |
| August 21.......... | August 25 | 72.49 | 72.50 |
| | | 72.37 | 72.39 |
| September 1........ | September 6 | 72.50 | 68.15 |
| | | 72.22 | 68.07 |
| September 14....... | September 21 | 68.92 | 65.15 |
| | | 68.54 | 65.95 |
| September 27....... | October 4 | 69.09 | 67.48 |
| | | 68.82 | 67.04 |
| October 25.......... | October 30 | 65.80 | 63.62 |
| | | 65.66 | 63.75 |
| October 25.......... | November 23 | 68.24 | 60.92 |
| Cured.............. | .............. | 68.01 | 60.95 |

apparatus, but drawn through a calcium chloride tube and then through sulphuric acid, to supply a dry atmosphere in the other. Table VI shows data for this experiment. These data show that

TABLE VI

EFFECT OF CURING ON MOISTURE CONTENT OF TRIUMPH VARIETY

| PERCENTAGE MOISTURE IN FRESH MATERIAL | PERCENTAGE MOISTURE AFTER 10 DAYS AT 30° C. | | | |
|---|---|---|---|---|
| | Dry atmosphere | Loss | Moist atmosphere | Gain |
| 68.15..................... | 67.20 | .......... | 71.76 | .......... |
| 68.07..................... | 67.30 | .......... | .......... | .......... |
| Average 68.11..................... | 67.25 | 0.86 | 71.76 | 3.65 |

there is not much gain or loss in the moisture content as influenced by that of the atmosphere.

CURING TEMPERATURE AND REGENERATION.—In order to determine whether regeneration during the curing process was due to the decrease in moisture content, or to the higher temperature main-

tained during the process, sweet potatoes were placed in moist and dry atmospheres at the usual curing temperature. These atmospheres were continually being changed by continuous streams of air maintained by a suction pump, as already described. Results showed that roots and shoots both developed in the moist atmosphere, while shoots only, or no growth at all, occurred in the dry atmosphere. It is evident, therefore, that the higher temperature is the main external factor in promoting growth, and that lack of moisture retards growth. The appearance of shoots is not necessarily a good index for complete curing.

HYDROGEN-ION CONCENTRATION OF EXPRESSED JUICES.—Determinations, by means of the hydrogen electrode, were made at

TABLE VII

HYDROGEN-ION CONCENTRATION OF EXPRESSED JUICES

| DATE OF HARVEST | DATE OF DETERMINATION | PORTO RICO | | | TRIUMPH | | |
|---|---|---|---|---|---|---|---|
| | | Milli-volts $P_D$ | Tem-per-ature | $P_H$ | Milli-volts | Tem-per-ature | $P_H$ |
| August 21............ | August 25 | 619.8 | 24.5 | 6.33 | 620.6 | 25 | 6.34 |
| September 1.......... | September 10 | 612.4 | 22.0 | 6.23 | 608.8 | 22 | 6.17 |
| September 14......... | September 21 | 612.6 | 21.0 | 6.26 | 609.0 | 22 | 6.17 |

various intervals. Table VII shows data for these determinations. The data show that there is not much difference in the hydrogen-ion concentration of the expressed juices of the two varieties. This is most likely due to the buffer action of the various substances present. Some proteins were included, since the juice was separated from the pulp by pressing it through a cloth.

CATALASE ACTIVITY.—A number of determinations of catalase activity have been made. In every case when the material had been under the same conditions of treatment, the Porto Rico variety gave higher catalase activity. In preliminary experiments with material which had deteriorated in storage, curves for catalase activity obtained were very irregular. Table VIII shows data for normal tissue. These data show that both moisture and catalase activity are higher in Porto Rico variety than in Triumph.

AMYLASE ACTIVITY.—WOHLGEMUTH's method was used and found to be very convenient for comparative purposes. A test was also made to determine the influence of varying the hydrogen-ion concentration on amylolytic activity under the conditions of the

TABLE VIII

CATALASE ACTIVITY OF UNCURED SWEET POTATOES HARVESTED DURING SEPTEMBER AND OCTOBER

| DAYS SINCE HARVEST | PORTO RICO | | | | TRIUMPH | | | |
|---|---|---|---|---|---|---|---|---|
| | Weight of tissue (gm.) | O₂ liberated (cc.) | Rate per gm. | Percentage HOH | Weight of tissue (gm.) | O₂ liberated (cc.) | Rate per gm. | Percentage HOH in tissue |
| 13............. | 0.1283 | 13.96 | 107.5 | 72.3 | 0.2807 | 28.01 | 99.79 | 68.11 |
| 8............. | 0.3181 | 46.16 | 176.5 | 68.7 | 0.3891 | 18.27 | 46.96 | 65.55 |
| 9............. | 0.3230 | 37.90 | 117.3 | 68.9 | 0.3990 | 40.37 | 117.17 | 67.27 |
| 10............. | 0.2298 | 38.87 | 196.1 | 65.7 | 0.3145 | 26.94 | 85.66 | 63.68 |
| Average........ | 0.2498 | ....... | 149.3 | ....... | 0.3458 | ....... | 87.39 | ....... |

experiment. Three indicators, Congo red, litmus, and phenolphthalein were used to prepare media with three different $P_H$ values, approximately 5, 7, and 9. Amylase activity was found to be the same in the last two, but less active in the first, with a $P_H$ value of approximately 5. Several additional determinations were made with material in varying conditions of storage, curing, etc. Results showed values as high as 12 for amylolytic activity in some cases.

TABLE IX

AMYLASE ACTIVITY OF SWEET POTATO EXTRACT, JANUARY 5

| PREVIOUS TREATMENT | PORTO RICO | | TRIUMPH | |
|---|---|---|---|---|
| | Amylase extract (cc.) | $D^{38°}_{30'}$ | Amylase extract (cc.) | $D^{38°}_{30'}$ |
| Harvested October 25, cured....... | 0.6 | 8.33 | 1 | 5 |
| Harvested September 14, uncured... | 0.6 | 8.33 | 1 | 5 |

LACCASE.—This oxidase enzyme was shown to be present in both varieties by means of treatment with tetramethylparaphenylendiamine hydrochloride, and qualitative-quantitative tests were made with guaiacum on material at different times of harvest, after it had

been in storage 3.5–5 months. Table X shows data for these deter-
minations. While the guaiacum test is only qualitative-quantitative,
the data indicate that laccase had been inactivated, due to condi-
tions initiated by the heavy frost which occurred January 1, or the
organic peroxide which functions with peroxidase had been destroyed.

ABSENCE OF TYROSINASE.—By redissolving the alcoholic pre-
cipitate from the aqueous extract of each variety, and by testing it
with a suspension of tyrosin, no apparent discoloration occurred
even after twelve hours, while in the control containing an aqueous
solution of tyrosin a slight pink color developed. As a check on the

TABLE X

LACCASE AND PEROXIDASE ACTIVITY DETERMINED BY BLUING OF GUAIACUM,
APRIL 14, 1922; FIRST KILLING FROST, JANUARY 1

| DATE OF HARVEST | PREVIOUS TREATMENT | TIME IN SECONDS FOR BLUING OF GUAIACUM | | DATE OF HARVEST | PREVIOUS TREATMENT |
| | | Porto Rico | Tri-umph | | |
| --- | --- | --- | --- | --- | --- |
| October 12....... | None | 5 | 3 | October 12 | None |
| November 30.... | None | 5 | 6 | November 2 | None |
| December 7...... | None | 4 | 4 | December 7 | None |
| December 7...... | Tops removed October 5 | 3 | 4 | December 7 | Topped October 5 |
| January 11...... | Topped November 9 | 10 | ...... | ............. | ................ |
| January 11...... | No treatment | 10 | ...... | ............. | ................ |

method, an alcoholic precipitate from the juice of the Irish potato
was prepared, redissolved, and tested in exactly the same way with
a tyrosin solution. In a short time a pink color developed which
was followed with black. Furthermore, a preparation from the Irish
potato known to contain tyrosinase (4) was added to the juice and
pulp of the two varieties. No apparent discoloration occurred, indi-
cating that tyrosin does not occur in the sweet potato varieties
tested.

PEROXIDASE.—Qualitative tests were made for peroxidase by
treating the tissue with guaiacum and hydrogen peroxide. Oxidation
of the guaiacum to guaiacum blue under these conditions was more
rapid in every case, indicating the presence of peroxidase.

Further tests were made to distinguish laccase from peroxidase.

BACH'S (3) method for distinguishing laccase from tyrosinase suggested a way for distinguishing laccase from peroxidase. Laccase is thermostable at 70° C., while the organic peroxide which is necessary for the action of peroxidase without the addition of hydrogen peroxide is thermolabile at 60° C., or at 55° if the sweet potatoes are placed in an oven for several hours at the latter temperature. Table XI shows data for several determinations.

The data in table XI suggest an oxidase system which would account for discoloration. This system consists of laccase, which activates the oxygen of the air directly or by forming a temporary

TABLE XI

TESTS FOR LACCASE, PEROXIDASE, ORGANIC PEROXIDE, AND TANNIN IN PORTIONS
OF PORTO RICO SWEET POTATO; − = NEGATIVE TEST; * = POSITIVE;
** = VERY STRONGLY POSITIVE

| PORTION USED | TEST FOR LACCASE | | TEST FOR PEROXIDASE | ORGANIC PEROXIDE TEST, KI, STARCH, CH₃COOH | TANNIN TEST WITH DILUTE FeCl₃ |
|---|---|---|---|---|---|
| | Tetramethyl-p-phenylen-diamin HCl | Guaiacum | Guaiacum plus H₂O₂ | | |
| Fresh juice, healthy..... | *** | * | ** | * | ** |
| Same, boiled............ | − | − | − | − | ** |
| Fresh juice, wilted....... | ** | * | ** | ** | ** |
| Same, boiled............ | − | − | − | − | ** |
| Control HOH........... | − | − | − | − | − |
| Slices, healthy.......... | * | * | ** | * | * |
| Same, wilted............ | ** | ** | ** | * | *** |
| Heated at 55° C. six hours | * | * | ** | − | * |

peroxide independent of the organic peroxide which worked in conjunction with peroxidase. The chromogen involved is probably a tannoid substance.

PHYSIOLOGICAL GROUPING.—The data in tables I, III, IV, V, VIII, XI, and figs. 2 and 3 suggest that there is a physiological grouping. The Porto Rico variety has higher respiratory activity, its moisture content is higher, and those enzymes which were studied quantitatively were more active in every case. This suggested grouping corresponds to the grouping of varieties found by STEENBOCK and SELL (29), which was based on fat-soluble vitamines referred to under Methods. Porto Rico variety represents the group that is high in fat-soluble vitamines.

## Discussion

The changes which take place during the curing of the sweet potato are largely physiological, marked mostly by an increase of respiration for a few days after harvesting until a maximum is reached and then a gradual falling off to a nearly constant rate. These changes seem to take place regardless of the temperature, within reasonable limits, but the process is augmented by a temperature of 30° C., which is commonly used. An appreciable loss in weight occurs during the curing process, due to respiration accompanied by conditions favorable for the removal of carbon dioxide and excess water. The moisture content remains about the same, usually with a slight loss. Data in table V show a possible loss of 3.2 per cent from Porto Rico and 2.33 per cent from Triumph variety. In the interpretation of such data, it is necessary to take into consideration the fact that the sweet potatoes were subjected to uncontrollable environmental conditions in transit of approximately 900 miles by express. More exact data in table VI indicate a much smaller loss of moisture during the curing process than ordinarily believed. HASSELBRING (11) found for Big Stem a loss of 0.51 per cent, and for Southern Queen 3.28 per cent of moisture during the curing process followed by a few days in storage. He found that sugars increase and starch decreases during this same period.

Respiration in the sweet potato is relatively high as compared with that of the Irish potato, especially when the latter is in a dormant condition. Curing finally decreases the rate of respiration in the former. This might account for the increase in total sugars as found by MAGOON and CULPEPPER (18). Just as respiration decreasing at low temperatures is accompanied by accumulation of sugars (21), so the accumulation of sugars during the curing process may be related to the final decrease in respiration.

STICH (30) showed that by cutting the Irish potato in four parts, a considerable increase in the rate of respiration followed. MAGNESS (17) and SHERMAN (27) raised the question as to what extent respiration under those conditions is due to facilitating the exchange of gases, and to what extent it is due to direct stimulation. Data in table II show that the increase is largely due to the former. This also has a bearing on the explanation of shrinkage under commercial handling.

The discoloration mechanism, according to the data presented, seems to be a two-phase oxidase system. One phase consists of peroxidase and an organic peroxide comparable with that described by OVERHOLSER and CRUESS (22) in their study of the browning of apple tissue, and earlier described by KASTLE and LOEVENHART (13). The other phase seems to be another enzyme, laccase, which activates oxygen without the presence of the same organic peroxide of the first phase. RACIBORSKI (26), in his studies of sugar cane, found that after heating the cane to 60° C. no action followed with guaiacum, unless hydrogen peroxide was added. This would suggest a one-phase peroxidase organic peroxide system, in contrast with the two-phase system found in the sweet potato.

Very little evidence has been presented regarding the chromogen which is oxidized in the system. $FeCl_3$ indicated the presence of a tannoid substance. KOHMAN (14) attributes the discoloration of canned sweet potatoes to the action of iron on tannin compounds forming an insoluble substance.

The relatively large intercellular spaces may undoubtedly facilitate exchange of gases. The more rapidly respiring Porto Rico variety was found to have a higher percentage of carbon dioxide in the intercellular spaces. MAGNESS (17) found that by the removal of a part of the epidermis of the apple, there followed a decrease in the carbon dioxide of the intercellular spaces, most likely due to the facilitation of gaseous exchange.

In consideration of the influence of higher temperatures on the rate of respiration, BLACKMAN'S (5) temperature effect has been referred to, that when a process is determined as to its rapidity by a number of separate factors, the rate of the process is limited by the pace of the slowest factor. $Q_{10}$, or the temperature coefficient for respiration in sweet potatoes, ran a little low for ARRHENIUS' temperature law for chemical reactions, where the coefficient is between 2 and 3. The coefficient for respiration in sweet potatoes is more nearly that of a heterogeneous system where two processes occur, the one retarding the other. GORE (8), however, found respiratory coefficient in fruits to be 2.38.

With respect to regeneration in the sweet potato, it is commonly known that adventitious buds arise from the stem end of the roots which are used for propagating. McCALLUM (15) defined regenera-

tion as the replacement of an organ or structure that has been re-
moved. The shoots referred to on the sweet potato are potentially
new individual plants. That they are formed during curing seems to
be significant in showing that the dormant period in this case de-
pends on lower temperature.

It has been commonly recognized that Porto Rico variety is
more popular in northern markets because of its edible qualities. It
is soft fleshed, and seems to be sweeter than Triumph. Physio-
logical grouping of varieties is suggested by the greater respiratory
and enzymatic activity of the former variety as compared with the
latter. This distinction is comparable with STEENBOCK and SELL's
grouping, based on fat-soluble vitamine content. Porto Rico repre-
sents the orange-yellow fleshed varieties, while Triumph represents
the white fleshed varieties. When they did their work, it was thought
that possibly the yellow pigment in the former variety might act as
a growth promoter. This was investigated by PALMER (23), and
found to have no significance.

## Summary

1. Freshly dug sweet potatoes, varieties Porto Rico and Tri-
umph, increase in their rate of respiration apparently from the date
of harvest until a maximum is reached, and then the rate gradually
falls to a nearly constant level.

2. Cured sweet potatoes do not reach so high a maximum rate of
respiration under the same conditions of storage and experimental
procedure.

3. Curing decreases the rate of respiration.

4. Increase of respiration as a result of injury when the cortical
layer is broken is largely due to facilitating the exchange of gases,
rather than to direct wound stimulation.

5. Curing under carefully controlled conditions does not reduce
the moisture content to any great extent.

6. Porto Rico variety has a larger percentage of carbon dioxide
in the intercellular spaces than Triumph under the same conditions
of storage and treatment.

7. Respiration in both varieties responds to each 10° rise in
temperature in much the same way between 15° and 35° C., having

nearly the same respiratory coefficients. The former variety respires at the greater rate.

8. Regeneration frequently occurs during the curing process, which indicates that dormancy depends on the existence of lower temperature, approximately 25° C. and lower.

9. The hydrogen-ion concentrations of the expressed juices are about the same in each variety, being slightly acid.

10. Moisture and catalase activity are higher in Porto Rico variety than in Triumph.

11. Both laccase and peroxidase are present in Porto Rico variety. These enzymes seem to be important in connection with discoloration of the tissues on exposure to the air.

12. Laccase is thermostable at 60°–70° C., while the organic peroxide of the peroxidase-oxidase system is thermolabile at 60°–65° C.

13. The oxidase system seems to be injured by changes initiated by chilling.

14. Amylase activity varies considerably with storage conditions and treatment. Optimum activity occurs in a medium with a $P_H$ value of 7–9, with a marked falling off at $P_H$ 5. Amylolytic activity of Porto Rico is greater than that of Triumph.

15. Tests for tyrosinase were negative in each variety.

16. A physiological distinction of the two varieties is evident. Porto Rico has greater respiratory activity, greater enzymatic activity, and a higher moisture content.

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# TRANSECT METHOD OF STUDYING WOODLAND VEGETATION ALONG STREAMS

J. E. WEAVER, HERBERT C. HANSON, AND JOHN M. AIKMAN

(WITH ELEVEN FIGURES)

In pursuance of an investigation of competition among certain shrubs (*Rhus glabra*,[1] *Corylus americana*, *Symphoricarpos* spp.), and between these shrubs and *Quercus macrocarpa*, it became imperative, for a proper understanding of their behavior, to ascertain just how near the edge of their range they were growing. The station, which is located at Weeping Water, Nebraska, is on the Weeping Water River, a branch of the Missouri, 15 miles west of its mouth. This stream arises 15 miles farther west, and in general its course parallels that of the Platte River about 15 miles north. As is generally the case throughout eastern Nebraska, chaparral and woodland are confined to the vicinity of the stream courses. Rather than following the meandering stream throughout its length, it was decided to make transects at six or seven places, thus saving much time during a busy field season. The results were so interesting, and the method so satisfactory in vividly portraying distribution with change of habitat, that it was repeated on the Little Nemaha River, a neighboring stream of similar size and general direction of flow. Unlike the Weeping Water, which cuts a deep canyon, this stream has a broad flat floodplain.

The initial transect was made at the headwaters, about 2 miles northeast of Eagle. Here the unbroken (but pastured) prairie sloped to form a broad valley, in only the lower part of which an intermittent stream, dry nearly all summer, had begun to cut a channel. *Salix nigra* occurred as isolated individuals or in intermittent clumps (fig. 1). Aside from a few *S. amygdaloides* and *Populus deltoides*, no other trees were represented, and there were no shrubs. Similar vegetation prevailed for two miles, although the channel became well defined, often with steep banks 4 or 5 feet high.

[1] Nomenclature is according to BRITTON'S *Manual of the flora of the northern states and Canada.* 2d ed.
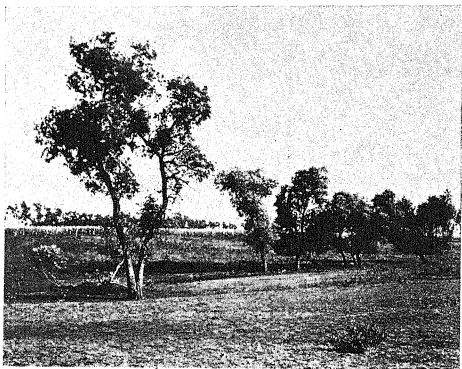
FIGS. 1, 2.—Fig. 1, willows (*Salix nigra*) at headwaters of Weeping Water River; fig. 2, development of stream in transect 3; elms and cottonwoods characteristic trees.

The second transect, which, like the first, included nearly a mile of the stream course, showed marked changes. The spring-fed stream ceased to be intermittent. It had cut banks 10–20 feet wide, and in places 8–10 feet deep, through the broad, prairie covered valley. In a few meanders, broad, somewhat wind protected sloping banks had been formed above the general channel. These were favorable places for a scattered growth of shrubs, chief among which were *Symphoricarpos symphoricarpos*, *S. occidentalis*, *Sambucus canadensis*, *Prunus americana*, and *Amorpha fruticosa*, the last growing down to the water's edge. *Ribes gracile*, *Rhus glabra*, *Celastrus scandens*, and *Vitis vulpina* were also found, but they were rare. *Salix nigra* was dominant, with considerable *S. amygdaloides*, the trees being much larger than before (some 2 feet in diameter) and much more abundant. A single small specimen of *Fraxinus lanceolata* and two of *Prunus virginiana* were the only other woody plants.

A third transect 2 miles beyond and just northwest of Elmwood gave a surprisingly large number of species. Here the creek, augmented by the water from laterals, was 2–8 feet wide, and had a distance of 30–45 feet between the sloping banks, which were 12–15 feet high (fig. 2). The trees were no longer confined to the banks, but also sometimes occupied the floodplain bordering the channel for a distance of a few yards (fig. 3). They were *Ulmus americana*, *U. fulva*, *Acer Negundo*, *Fraxinus lanceolata*, and *Salix nigra*, the last in much fewer numbers than formerly. All the preceding frequently reached diameters of one and sometimes two feet. *Populus deltoides* was also quite abundant, the largest trees being 3–4 feet thick. *Juglans nigra* was found sparingly, only a few well developed trees occurring in the mile transect. Small trees of *Morus rubra* were infrequent. The more favorable conditions were further shown by the extensive growths of *Symphoricarpos occidentalis* and *S. symphoricarpos*, both on the banks and over the floodplains, where thickets 6 feet high were sometimes found. *Ribes gracile*, *Sambucus canadensis*, and *Amorpha fruticosa* were very common in both situations. *Cornus asperifolia*, *Celastrus scandens*, and *Smilax hispida* were less frequent, but *Vitis vulpina* was very common, sometimes with stems 3 inches thick. *Rhus toxicodendron* was common, *Prunus ameri-*

*cana* less so; *Rhamnus lanceolata* and *Rubus occidentalis* were also found. In no place was the woodland belt more than a few rods wide.

In one portion of the transect, the stream in changing its course had left a south bank, 15–30 feet high, with a small pond near its base. Through this bank there entered a steep sided ravine, the whole offering a protected place for tree growth. On the sides of this ravine, and on the protected north-facing bank was found a scattered growth of *Quercus macrocarpa*, consisting of small trees mostly 5
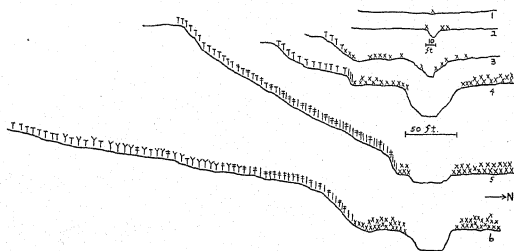


FIG. 3.—Profiles of Weeping Water valley, where transects 1–6 were made: nos. 3–6 show only the south slopes bordering the valley; X, floodplain dominants; T, bur oak; l, linden; ‡, red oak; Y, shellbark hickory.

inches or less in diameter, and perhaps 35 feet in height. Associated with it was *Hicoria minima* of similar size, while on the protected lower slope and floodplain were fine specimens of *Celtis occidentalis* and *Gleditsia triacanthos*. *Prunus serotina* was also represented, but by small trees.

Quite in contrast with this area were stretches of the unprotected, windswept valley, which were nearly or quite destitute of woody vegetation. No *Corylus americana* was found, but this occurred quite abundantly along with the oak just northeast of Elmwood.

A fourth examination 3 miles farther down stream and just southwest of Wabash showed further development. Forest growth

extended well over the eighth-mile wide floodplain, which was some-
times bordered on the south by steep slopes 20–50 feet high, covered
with upland trees. Dense growths of *Acer Negundo* and *Ulmus*
clothed the lowlands subject to overflow. The floodplain forest
proper was composed of red and white elm, green ash, walnut, and
box elder, and occasionally willows and cottonwoods. Bordering
this, especially on steeper banks, were well developed specimens of
*Tilia americana*, some 17 inches thick, elms, bur oak, and a little
hickory. The oak especially, and the hickory in smaller numbers,
spread up ravines and to the crests of hills, forming woodland to a
distance of one-fourth mile from the stream (fig. 3). The increasing-
ly favorable growth conditions were revealed in many ways. The
floodplain trees were much more abundant, taller, of greater diam-
eter, and the stands were denser. Walnut, ash, and elms, 1–1.5
feet in diameter, were frequent, some of the elms being 4 feet thick.
Hackberry, choke cherry, and honey locust, while not abundant,
were found scattered throughout, while *Gymnocladus dioica* ap-
peared for the first time. The linden, at first represented by small
trees, became 10–12 inches in diameter in more favorable situations.
Its range of habitat was very limited, however, being confined to the
lower portions of steep slopes (fig. 3). Many of the oaks were 1.5 feet
and some 2.5–3 feet in diameter. The wider range of *Ribes gracile*
far back from the stream, together with its greater abundance and
greater stature, is significant. *Symphoricarpos* spp. formed a shrubby
undergrowth, alternating with thickets of *Corylus* in more favored
and not too shady situations, and extended beyond the fringing oak
forest. These, and *Cornus, Sambucus, Rhamnus, Amorpha,* and
*Prunus americana* showed a more vigorous growth, as did also the
lianas, *Vitis, Celastrus,* and *Rhus toxicodendron,* which sometimes
climbed over the shrubs. *Sambucus* was especially abundant. *Rhus
glabra* fringed the floodplain forest and extended far into the upland.
The following shrubs and vines were also present, the first only in
abundance: *Smilax hispida, Parthenocissus quinquefolia, Xanthoxy-
lum americanum, Rubus occidentalis,* and *Clematis missouriensis.*
These indicate the rapid development toward true forest conditions.
    Four miles eastward the river begins to cut a canyon in the
Pennsylvanian (Carboniferous) limestone. Just east of Weeping

Water, 7 miles from the fourth transect, this reaches a depth of 120 feet. Here it is joined by Cascade Creek, also in a small canyon. Taking advantage of the shelter afforded by the rough topography, trees and shrubs have spread widely from the streams, and form a belt of timberland more than a mile wide. Only on the drier, wind-swept southwest slopes does the prairie hold forth, and experimental evidence shows that it is giving way slowly to shrubs and trees (fig. 4). Indeed, the habitat is so diversified that a distinct grouping of the members of the woodland community into associes has occurred. On the floodplains a mixed elm-walnut-ash associes is found; the lower slopes of the canyon banks are clothed with forests of red oak
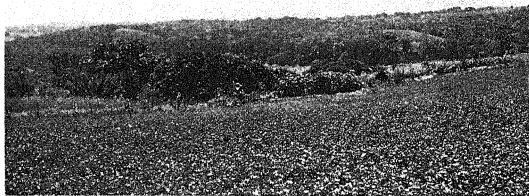


FIG. 4.—General view of woodland near Weeping Water (transect 5), showing grassland on thin soils overlying limestone on exposed southwest slopes.

and linden; the higher, as well as the protected south slopes, are clothed with bur oak and hickory. The last is fringed on its upper margins with a chaparral community, which extends under the open forest as a more or less suppressed layer. Upon the removal by cutting of any of these forest types (fig. 3), chaparral immediately springs into dominance.

FLOODPLAIN ASSOCIES.—In this transect the floodplain is nowhere over one-third of a mile wide, in fact often less, but much better protected than heretofore. Fine specimens of red and some white elm, walnut, and green ash, 25–50 feet tall and 10 to over 18 inches in diameter dominate (fig. 5). Hackberry, box elder, and wild black cherry also make an excellent growth, while honey locust and Kentucky coffee tree are of less abundance. The chief shrubs are

species of *Symphoricarpos*, gooseberry, wild black raspberry, and
elderberry; while grape, smilax, bittersweet, and Virginia creeper are
the characteristic vines. All are well developed. The soil of the
floodplain forest is not especially rich in humus, since it is subject
to overflow, and attendant conditions of aeration or subsequent
drought exclude the growth of red oak and linden. Even a slight
rise in topography is favorable to these more exacting trees, how-
ever, and the floodplain forest often gives way abruptly to them.

RED OAK-LINDEN ASSOCIATION.—*Quercus rubra*, not occurring in
the preceding transect, here forms with *Tilia americana* a well defined
association. Trees with diameters 8–15 inches and about 50 feet tall
are common. The annual precipitation has increased 2 or 3 inches
as compared with that at the headwaters, humidity is much higher
on these wind protected slopes, and soil moisture is more abundant.
Humus is well developed, and rather typical mesophytic forest floor
conditions have been attained (fig. 6), *Ostrya virginiana* being the
characteristic secondary species. *Ulmus fulva* is common, especially
nearer the floodplain, where *Celtis*, *Juglans*, and other floodplain
species may occur infrequently. The density of the shade is shown
by dead or attenuated bur oaks or hickories, which species flourish
farther up the slopes. *Euonymus atropurpureus*, *Staphylea trifolia*,
and *Xanthoxylum americanum* occur, with certain other shrubs, but
as a whole undershrubs are few and layering is not well developed.
The width of this forest community is variable, depending upon the
slope, perhaps nowhere over 8 or 10 rods. Just as the lindens grow
best on the lower banks, the red oak more nearly dominates farther
up the slopes, and the transition to the bur oak-hickory community,
although often abrupt, may be quite gradual.

BUR OAK-HICKORY ASSOCIES.—This community was represented
almost entirely by open growths of *Quercus macrocarpa*, *Hicoria
minima* not being at all important (fig. 7). The trees were usually
6 inches or less in diameter, and only 35–40 feet tall, but excellent
specimens were found in favored sites, forming an open woodland.
The crowns were rather open. About half the forest was carpeted
with *Poa pratensis;* the denser part was covered with a good leaf
mulch, the grasses having been shaded out. Aside from a very few
scattered hickory and an occasional box elder, with red elm or red
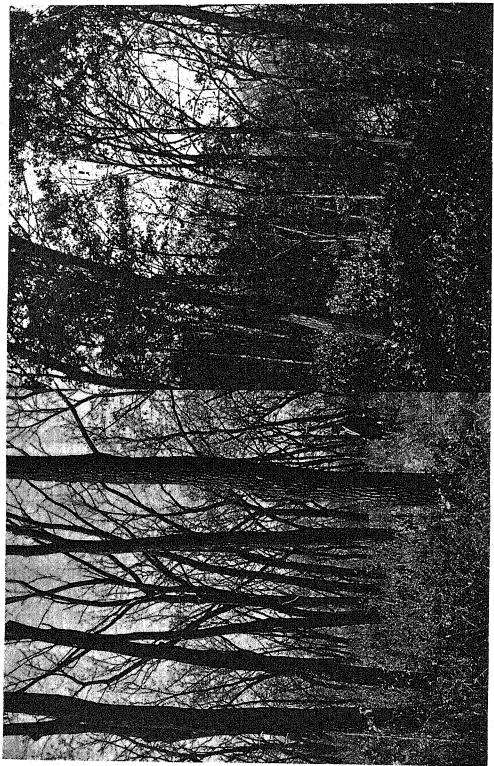
FIGS. 5, 6.—Fig. 5, floodplain forest of ash, elm, and walnut at Weeping Water; fig. 6, red oak-linden forest on steep north slope.

oak in the moister areas, practically no other trees occurred. *Corylus*, *Symphoricarpos*, *Rhus glabra*, and *Cornus asperifolia* were the characteristic undershrubs, although *Rhamnus lanceolata*, *Sambucus canadensis*, and *Cornus stolonifera* also occurred. The first was found only near the forest margins, or in extensive, dense thickets on cut-over areas. *Symphoricarpos*, often poorly developed, was characteristic throughout; *Cornus* made its best growth in the open places or near the forest margins; while sumac was characteristically a fringing shrub, not enduring the shade well.

CHAPARRAL ASSOCIES.—In the chaparral proper *Corylus* showed its greater mesophytism by occupying the more sheltered areas, although it sometimes occurred on protected hilltops. *Rhus glabra* was more often found on the rocky hilltops and exposed slopes, while *Symphoricarpos symphoricarpos* and *S. occidentalis* occupied a somewhat intermediate position, but showed striking xerophytic tendencies. Associated with these shrubs, especially with the hazel, were *Cornus asperifolia*, *Rhamnus lanceolata*, *Ribes gracile*, *Xanthoxylum americanum*, and various lianas, especially *Celastrus scandens* and *Smilax hispida*, the whole often forming a dense tangle over extensive areas. The shrubs invade the grassland chiefly by their rhizomes and other methods of vegetative propagation. The grasses gradually succumb to these woodland outposts, because of unfavorable light relations, due in part to accumulations of débris. Forests of bur oak may follow in their wake. Thus most of this transect showed continuous woodland cover; ash-elm-walnut forests on the floodplains, red oak and linden on the well protected deeper soils of the north-facing slopes, bur oak in the drier soils above, perhaps with chaparral near and on the hilltops, giving way again to bur oak on the protected lower south-facing slopes, with red oak and linden in the ravines. As a whole the forests are young, however, and grassland often holds forth on the thin soils overlying the limestone on the exposed and windswept south and southwest hillsides (fig. 4).

The next transect was made about 8 miles farther eastward, one and one-half miles beyond Nehawka. Because of its proximity to the forests along the Missouri River, only 6–8 miles farther east, and increasingly favorable growth conditions, the associes were better developed. In fact woodland would undoubtedly be continuous ex-

FIGS. 7, 8.—Fig. 7, interior of young bur oak forest; fig. 8, floodplain forest of ash, elm, hackberry, and walnut near Nehawka (transect 6).

cept for fires, grazing, cultivation, or other disturbances. The Weeping Water, augmented by numerous tributaries, here assumed the proportions of a rather large stream. The bed is 10–15 feet wide, and in spring and early summer carries a rather large volume of water. Owing to the absence of deep canyons, it has a broad floodplain, extending in places nearly a mile across the valley floor.

The floodplain forest, as before, consisted largely of red elm, ash, and walnut; and hackberry, Kentucky coffee tree, and cottonwoods played an important part. The trees were much taller and of greater diameter than at Weeping Water (fig. 8). Among the dominants a diameter of 16–20 inches was not uncommon, and specimens of elm and hackberry over 3 feet in diameter, breast high, were noted. Fine specimens of walnut logs from this area measured 18–32 inches in diameter, and varied in age from 60 to 170 years. Several specimens of *Gymnocladus* 17 inches thick were found. Other trees characteristic of the floodplain were *Salix nigra, S. amygdaloides,* and *Gleditsia triacanthos,* large trees, but rather infrequent; *Acer saccharinum, Acer Negundo, Prunus serotina,* and *Crataegus mollis,* all rather infrequent.

On higher ground, and especially on north-facing slopes, the floodplain forest gave way to that of red oak and linden. As in the preceding transects, the linden grew best on steeper slopes, where fine, clean boled trees 60 or more feet tall and 10–20 inches in diameter occurred. It was not so limited in its range as heretofore, but extended well up over the gentle slopes, where with red oak it formed a dense forest canopy. Aside from ironwood other trees were rare. The deep litter of leaves and duff covered a rather mellow black soil, filled with fungus mycelia, but supporting only a scant shrubby and herbaceous vegetation of shade enduring species (fig. 9). This forest belt was of variable width, depending upon slope protection. On higher land, the dropping out of the linden was counterbalanced by the appearance of the shellbark hickory, which for a distance afforded a mixed red oak-hickory forest. Higher up the slopes, however, this soon gave way to nearly pure growths of hickory, which covered extensive areas (fig. 10). The trees were mostly 7–10 inches in diameter. This forest was interposed between the red oak-linden association and the bur oak community (fig. 3). Undergrowth
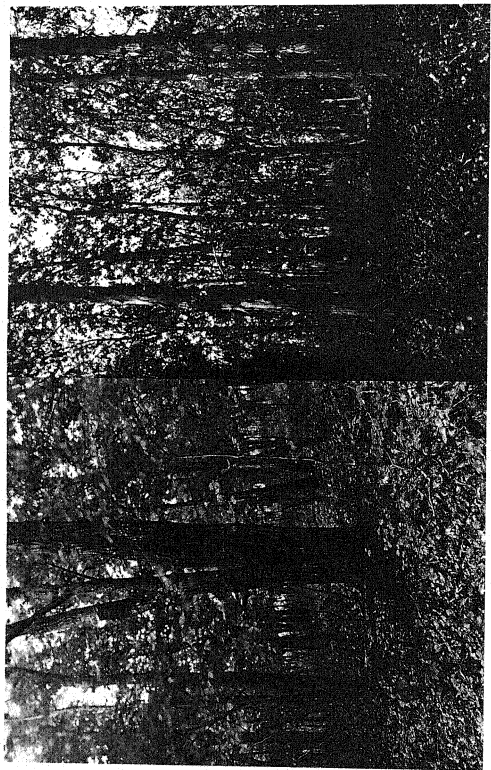
FIGS. 9, 10.—Fig. 9, well developed red oak-linden forest, with undergrowth of ironwood, near Nehawka; fig. 10, shell-bark hickory forest near Nehawka.

### TABLE I

<small>OCCURRENCE AND IMPORTANCE OF TREES AND SHRUBS ALONG WEEPING WATER RIVER (*D*, DOMINANT; *S*, SUBDOMINANT; *A*, ABUNDANT; *F*, FREQUENT; *I*, INFREQUENT)</small>

| SPECIES | TRANSECT | | | | | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 |
| **Trees** | | | | | | |
| Salix nigra | D | D | S | S | S | S |
| Salix amygdaloides | D | D | S | S | S | S |
| Prunus americana | | S | S | S | S | S |
| Prunus virginiana | | S | S | S | S | S |
| Ulmus americana | | | D | D | D | D |
| Ulmus fulva | | | D | D | D | D |
| Acer Negundo | | | D | D | S | S |
| Fraxinus lanceolata | | | D | D | D | D |
| Juglans nigra | | | S | D | D | D |
| Quercus macrocarpa | | | S | D | D | D |
| Populus deltoides | | | S | S | S | S |
| Morus rubra | | | S | S | S | S |
| Hicoria minima | | | S | S | S | S |
| Celtis occidentalis | | | S | S | S | D |
| Gleditsia triacanthos | | | S | S | S | S |
| Prunus serotina | | | S | S | S | S |
| Tilia americana | | | | S | D | D |
| Quercus rubra | | | | | D | D |
| Gymnocladus dioica | | | | S | S | S |
| Ostrya virginiana | | | | | S | S |
| Amelanchier canadensis | | | | | S | S |
| Salix interior | | | | | S | S |
| Hicoria ovata | | | | | | D |
| Quercus velutina | | | | | | S |
| Crataegus mollis | | | | | | S |
| **Shrubs** | | | | | | |
| Symphoricarpos symphoricarpos | | A | A | A | A | A |
| Symphoricarpos occidentalis | | A | A | A | A | A |
| Sambucus canadensis | | F | F | A | F | F |
| Amorpha fruticosa | | F | F | F | I | I |
| Ribes gracile | | I | F | A | A | A |
| Celastrus scandens | | I | I | F | A | A |
| Vitis vulpina | | I | A | A | A | A |
| Rhus glabra | | I | F | A | A | A |
| Cornus asperifolia | | | I | F | A | A |
| Rubus occidentalis | | | I | I | F | F |
| Smilax hispida | | | I | A | A | A |
| Rhus toxicodendron | | | F | A | A | A |
| Rhamnus lanceolata | | | I | F | A | A |
| Corylus americana | | | | F | A | A |
| Parthenocissus quinquefolia | | | | I | F | F |
| Xanthoxylum americanum | | | | I | F | F |
| Clematis missouriensis | | | | I | I | I |
| Euonymus atropurpureus | | | | | I | I |
| Staphylea trifolia | | | | | I | I |
| Cornus stolonifera | | | | | I | I |

in the two communities was not markedly different, although both the shrubby and herbaceous layers were better developed in the bur oak forest, where shade was less dense. *Symphoricarpos* was the chief shrub, although *Cornus* and *Corylus* occurred sparingly. Usually the leaf mulch and shade caused the disappearance even of blue grass, which formed a rather characteristic carpet in more open bur oak forest. The intolerance of the bur oak was often shown by dead trees only a few inches in diameter where the hickories occupied the territory.

Bur oak forest, often with a considerable admixture of bitternut hickory, also covered wide areas. Here, too, the trees were of greater stature than at Weeping Water, but the forest structure was very similar. Chaparral in cutover areas was very well developed. All of the species found in the preceding transect again occurred, usually in increased numbers, and their enumeration for the different communities is quite unnecessary. The almost total absence of *Quercus velutina* was rather surprising. In the better developed forests along the Missouri River southward at Peru, it occupies an intermediate position between the bur oak and shellbark hickory and red oak.

For several miles east of this transect, the widening floodplain has been cleared of timber and the land is cultivated, often quite to the banks of the stream; hence further study was not made. Such a clearing will form a rather effectual barrier to tree migrants up stream. The occurrence and importance of the various trees and shrubs in the several transects are summarized in table I.

## Studies along Little Nemaha River

The Little Nemaha has its headwaters (north fork) 2 miles southeast of Cheney and about 10 miles south and 10 miles west of those of the Weeping Water. Its southeastern course roughly parallels that of the latter, approximately 15–20 miles southward.

Conditions in the first transect were practically identical with those already described, *Salix nigra* alternating with *S. amygdaloides*, in a broad valley with practically no channel or merely the beginnings of a ditch.

A second transect, 2 miles eastward, was made across prairie land, where a meandering stream with banks 2–3 feet high was

usually dry in late summer. Low meadow and swamp land about 7 rods wide occupied a part of the area. The dominant tree was *Salix nigra*, which was fairly abundant and 8–14 inches in diameter, but *S. amygdaloides* of equal size was found. *Populus deltoides* was less abundant. *Amorpha fruticosa* and *Sambucus canadensis* were both quite abundant, the former attaining a diameter of 2 inches. *Vitis vulpina*, with a similar stem diameter, was found growing to a height of 30 feet on some of the willows. A single *Ribes gracile* also occurred. A few *Acer saccharinum* and *A. Negundo* had escaped from neighboring groves, as had also one or two *Gleditsia triacanthos* and *Fraxinus lanceolata*. All were small trees. A thicket of *Prunus americana* in a ravine on a neighboring hillside was fringed with *Rhus glabra*, and sheltered a few *Ribes gracile* and *Rubus occidentalis*.

Two and one-half miles eastward and just south of Bennett, a third examination was made. The stream here had a well defined channel, with a depth of 8 feet, with running or standing water, except in the driest times. Because of the gentle gradient, the valley was filled with ox-bow loops, so that from a distance the woodland appeared to be 8 or 10 rods wide. In reality it did not get far beyond the protecting stream banks, which sloped back gradually on depositing shores. A rather precipitous ridge 20–30 feet high bordered the floodplain on the south, and furnished much protection from the wind. *Salix nigra* had entirely disappeared, the dominant floodplain trees being *Fraxinus lanceolata*, *Acer Negundo*, and *Ulmus americana*. Secondary species were *U. fulva*, *Celtis occidentalis*, and *Gleditsia triacanthos*. The shrubs were *Symphoricarpos symphoricarpos* and *S. occidentalis*, about equally abundant. They formed intermittent areas on the lowland and extended well up on the high ridge. Scattered bushes of *Ribes gracile* were common throughout the lowland. *Vitis vulpina*, and to a less extent *Parthenocissus quinquefolia* were rather common. *Amorpha fruticosa* occurred sparingly. A deep, sheltered ravine, extending into the high bordering southern ridge, formed a locally protected area, in which were found *Cornus stolonifera*, *Euonymus atropurpureus*, *Prunus americana*, *Rhus glabra*, and *Rubus occidentalis*, all abundant; and *Clematis virginiana*, *Rhamnus lanceolata*, *Rhus toxicodendron*, *Prunus demissa*, and *Smilax hispida* in smaller numbers. The last was also found on the

floodplain. Near the lower end of the mile transect two small *Quercus macrocarpa* and a single *Juglans nigra* were observed. A mile below, at the junction of the three forks, bur oak was abundant.

A fourth transect was made 2 miles southwest of Palmyra, and about 6 miles east of the third transect. Here the stream had shallow running water 4–8 feet wide during the dry late summer. The rather steeply sloping banks were 8–20 feet high, while the floodplain varied from one-eighth to nearly one-fourth of a mile in width. The lower portion next the stream for a distance of 1–5 rods was subject to overflow, while much of the remainder had been given over to the cultivation of crops. The dominant floodplain trees were red and white elm and green ash, with an abundance of box elder in the more open parts especially subject to overflow. Black walnut and hackberry were both very abundant. When growing in fairly close stands fine specimens of elm, walnut, and hackberry, 50–60 feet tall and from 1 to over 2 feet in diameter, were not infrequent. Thus the dominants had increased one-third to one-half in height. Species of less importance were cottonwood (locally abundant), black willow, and honey locust. Both species of *Symphoricarpos* were abundant everywhere, indicating by their stature much more favorable growth conditions than formerly. Gooseberries, elder, raspberry, grape, Virginia creeper, poison ivy, and smilax were found throughout, all being well developed where the shade was not too dense. Dogwood and burning bush were also widely distributed.

A considerable portion of the floodplain was sheltered on the south by a bank extending 20–40 feet above the general level. Here *Quercus macrocarpa* and *Hicoria minima* formed almost pure stands. Many of the oaks were 6–10 inches in diameter, the hickory being slightly smaller and not getting so far up the hillside as the oak. Beneath the oak and hickory was a well developed layer of *Cornus asperifolia, Corylus americana, Ribes gracile,* and *Rubus occidentalis. Rhamnus lanceolata* was represented sparingly. *Symphoricarpos* spp. and *Rhus glabra* extended from the forest edge to form dense chaparral on the drier upper slopes, which were treeless. A few other trees were found, but none were large specimens. These were *Morus rubra, Prunus demissa* (somewhat abundant), *Gymnocladus dioica,* and *Aesculus glabra.* The last two were found in scattered small

clumps only in the most sheltered parts of the forest. Their pres-
ence indicated a considerable degree of mesophytism. This was
further shown by the invasion of elms, hackberries, and others into
the lower slope occupied by the bur oak and hickory.

Upon leaving this area the floodplain gradually widened to half
a mile, and then to over a mile in extent, the bordering hills thus
usually being far removed from the present tree belt (fig. 11). Two
factors had entered to disturb the forests. A drainage channel had
been cut in the valley from Syracuse to the Missouri River, a dis-
tance of approximately 40 miles, thus leaving much of the old
channel dry. The most destructive disturbance, however, had been



FIG. 11.—View along the broad floodplain of Little Nemaha below Syracuse

brought about by the clearing of the forests and the cultivation of
the broad fertile floodplain; in fact, it was usually tilled to within a
few rods of the stream. Hence much of the remaining vegetation was
on land subject to overflow, and only fragmentary sites of oak-
hickory were to be found. Extended examination of two transects,
the first one mile southwest of Syracuse and another just west of
Talmage (which is 25 miles southeast of the Palmyra transect), as
well as a third at an intermediate point, showed little change in the
floodplain species. As before, elms, ash, walnut, and hackberry were
most abundant, with box elders in the newer areas more subject to
overflow. Honey locust and willows often played an important rôle.
All were nearly or quite as well developed as in the most sheltered
portion of the preceding transect, some being even taller and of

greater diameter. Their general distribution over the floodplain, and the greater continuity of the forest, revealed more favorable conditions for growth. This was further shown by an excellent growth of bur oak and hickory on certain south-facing slopes above the floodplain. With these were found all the shrubs and lianas before enumerated as characteristic of the associes. Kentucky coffee trees and honey locusts were much larger than before, as were also the hickories on the higher floodplains. In general, all the shrubs and lianas characteristic of the floodplain had made a much more vigorous growth, a phenomenon especially prominent in bordering plum thickets. *Robinia pseudacacia* was the only new species found, and this was very local, having escaped from cultivation.

The river continues its southeastern course, meandering in a floodplain 1.5–3 miles wide. The land rises without precipitous bluffs to the old dissected loess plain. No extensive tree growth, except that on the uncultivated remnants of the floodplain, was seen for a distance of 10 miles below the last transect. Five miles southeast of Brock, where Rock Creek enters from the north, considerable tracts of undisturbed woodland were found. This stream, with a floodplain one-fourth mile wide, is bordered by hills which rise somewhat abruptly 50–60 feet.

The floodplain forest dominants, as at Weeping Water, were *Ulmus fulva, Fraxinus lanceolata,* and *Juglans nigra.* Secondary species and the undergrowth of shrubs were also similar. On the lower, moist slopes bordering the floodplain, a mixed forest of red oak, shellbark hickory, and black oak occurred. Only in limited areas on the longest slopes did a segregation into rather distinct communities occur. These, however, were sufficiently pronounced to show clearly the dominance of the red oak on the best developed, most mesophytic areas. The shellbark hickory, often intermixed with black oak, or the latter forming an upper portion to the black oak-hickory community, occurred above the red oak zone. These trees were 10–20 inches in diameter, and 45–55 feet in height. The higher slopes, even those quite exposed, were clothed with a continuous cover of bur oak and bitternut hickory, the latter being much more abundant than anywhere along the Weeping Water River. Shrubs forming the undergrowth in the woodland and fringing its

borders were of the same species, and quite as abundant as at the
Weeping Water station (transect 5). In general, the development of
the plant communities, owing to less shelter afforded by the more
regular topography, was poorer than at the latter station.

Twelve miles farther down the stream, and only 3 miles from its
junction with the Missouri, another favorable place for study was
afforded by the meandering stream, with its accompanying flood-
plain forest, flowing close to the foot of a rather steep north slope.
Avoiding undue repetition, it may be noted that here the forest com-
munities were of the same type and of similar distribution to those at
Nehawka. The trees and shrubs were fully as well developed, and
the black oak, which was rare at Nehawka, here played the rôle of
a dominant. Further studies would undoubtedly reveal other woody
species along the lower course of the Little Nemaha, but none of any
ecological significance.

### Discussion and conclusion

From these data it may be seen that a series of wide transects
made at carefully selected intervals along the course of a stream re-
veals in a striking manner the appearance of species in the general
sequence of their ecological requirements and their later develop-
ment into forest communities.

The pioneer trees at the stream sources are those with light,
windblown seeds, such as willow, cottonwood, elms, box elder, and
ash. Farther down stream, where a floodplain with protecting banks
occurs, trees appear which spring from large rodent-carried fruits,
such as walnut, bur oak, bitternut hickory, honey locust, etc. The
first shrubs and lianas to occur near the stream sources, with the
single exception of *Amorpha fruticosa*, all have showy edible fruits
which are readily carried by birds. Such are species of coral berry,
elder berry, gooseberry, bitter-sweet, grape, dogwood, raspberry,
greenbriar, etc. Cherry, plum, mulberry, and hackberry, all appear-
ing relatively early, migrate in a similar manner. Of course the
factor of migration, although an essential one, is of little significance
unless it is followed by eccesis, that is, germination, growth, and re-
production. Many forest trees, such as linden, red oak, and shell-
bark hickory, are clearly too demanding in their habitat require-
ments to pioneer against grasses, and migration alone would be of

little consequence. Chaparral and less mesophytic forests, such as bur oak, regularly precede them in their up-stream invasions.

A study of the sequence of the appearance of trees and shrubby species along stream courses originating in prairie, throws much light upon their ability to tolerate unfavorable growth conditions; for here the balance between forest and grassland is so delicate that a little higher water content, a slightly greater humidity, protection from drying winds, etc., throws the balance in favor of tree growth, while the reverse conditions exclude it. Rate of growth in the several transects, as revealed by width of annual rings, will add much to the story of adaptation to habitat, and form an excellent measure of the growth conditions in the several transects as integrated by the living plant.

At first invaders may appear in mictia, willow, box elder, elm, walnut, oak, hickory, and linden, all in the one rather undiversified habitat, the irregular floodplain. Soon, however, as the stream cuts its channel with a lower floodplain subject to overflow, a higher floodplain, and sloping banks and bluffs, the trees are promptly grouped into definite communities. The intolerant willows largely disappear; box elder clothes the lower floodplain; ash, elms, and walnut cover the upper; and linden, oaks, and hickories are found on the higher ground. Thus a stream course cutting deep canyons has the most diversified forests. One of the most interesting contrasts between the Weeping Water and the Little Nemaha was the rapidity of this early sorting into definite communities in the first, and the much greater distance traversed before this occurred along the latter stream. Well developed bur oak forests were found only 6 miles from the headwaters of the Weeping Water, but 11 miles down the Nemaha. Similarly, the red oak-linden community was well defined 18 miles from the headwaters of the former stream, but down stream a distance of 60 miles on the Little Nemaha.

The behavior of woodland species as revealed by this study, confirms the view that while the woodland along the upper portions of the streams is undoubtedly postclimax, that along their lower course is a part of the generally undeveloped woodland of the subclimax prairie.

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# PLANT COMMUNITIES OF ALPINE PARK

## W. G. WATERMAN

### (WITH FIVE FIGURES)

## I.  Environmental conditions

Alpine Park is situated in Glacier Park, Montana, on the summit of the Lewis Range just south of Logan Pass. The term park is used here in the same sense as elsewhere in the Rocky Mountains, meaning a broad, flat floored valley surrounded by mountain peaks. In this case the park is part of the floor of an upper cirque, which was formed by a glacier which flowed southeast through the valley of Reynolds Creek into the St. Mary's River. Post glacial erosion has reduced the area of the level ground, but it still has an extent of nearly one square mile.

The park is bordered on the north by Mt. Pollock, on the west by Mt. Oberlin and Mt. Clements, and on the southwest and south by the long ridge known as Mt. Reynolds (fig. 1). On the east the ground drops abruptly to the valley bottoms of two mountain streams, which unite to form Reynolds Creek. On the northwest the ring of mountains has been cut between Mt. Pollock and Mt. Oberlin by the recession of the head of Logan (Trapper) Creek Valley, and on the southwest by the cirque which now contains Hidden Lake. The continental divide crosses the park diagonally from Mt. Pollock to Mt. Clements, and then turns back at a sharp angle from Mt. Clements to Mt. Reynolds.  This is the result of the cutting back by the valley heads on the northwest and southwest, as a consequence of which the drainage from the northwest and southwest corners of the park flows into McDonald Creek and eventually to the Pacific Ocean. The rest of the run-off waters flow through Reynolds Creek and St. Mary's River to Hudson's Bay and the Arctic Ocean. A small glacier is located at the base of Mt. Clements on the western angle of the divide, so that its flanks extend over into the Pacific drainage area, and on this account it was named Two-

Ocean Glacier by LYMAN B. SPERRY, the pioneer explorer of part of
Glacier Park. The water from the central portion of the glacier
flows through a shallow valley across the north-central portion of
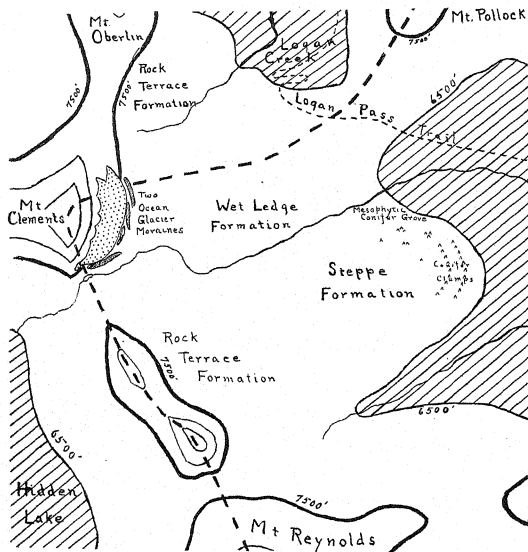


FIG. 1.—Map of Alpine Park: region described lies chiefly between the 6500 foot
and 7500 foot contours; areas below 6500 foot contour cross-hatched to bring out area
of park; dotted line indicates approximate line of continental divide.

the park, and discharges into a ravine cut between the main level of
the park and the slopes of Mt. Pollock on the north. At the south-
west corner of the Two-Ocean Glacier is a small pond situated on
the divide. A stream flows from either end of this pond, one joining
the stream which flows into St. Mary's Valley, the other flowing

into Hidden Lake and thence into the McDonald Valley. On this account the little body of water has been named Two-Ocean Pond.

TOPOGRAPHY.—The eastern portion of the park has a gently rolling topography, controlled by nearly horizontal rock ledges more or less covered by rock waste. On the northeast these ledges lie in concentric curves, but on the south and west sides they are straight, and parallel the bases of the mountains above them. On the west side of the stream valley the ground gradually rises in steps to the slopes of Mt. Oberlin and the moraines of Two-Ocean Glacier. Here the ledges are very marked, and their direction is slightly convex around the base of Mt. Clements. To the north and south of Mt. Clements they become larger and more prominent, and might be called rock terraces rather than ledges.

COMPOSITION OF SOIL.—The soil on the ledges is a thin coating of ground moraine, but in the depressions between them it is black and peaty. The moraines are made up of dry, powdery clay with a large admixture of angular gravel; in fact, they are probably composed partly of surface moraine in which the talus fragments have not been at all worn by glacial friction. There are no bowlder beds such as are found below Grinnel Glacier, owing to the absence of a high surrounding rock wall as a source of such material.

SOIL MOISTURE.—On the east the ledges are relatively dry, but the depressions hold slowly melting snowbanks, and the soil in them is peaty and wet. On the west side the depressions between the ledges are broad, and the dip of the beds is to the southwest, or against the flow of the surface water, so that the waters from the glacier and its surrounding snowbanks keep the soil water soaked most of the time. The rock terraces vary in the amount of water which they receive from above, those at the base of Mt. Oberlin being rather dry, while those on the south side are very wet, and the vertical faces of the terraces are always dripping with water.

CLIMATIC CONDITIONS.—The average elevation of the park floor is about 7000 feet above sea level, and it is always exposed to winds from the west and north, thus insuring a relatively low average temperature. This causes the moist air which flows up from the valleys to condense no matter what the direction of the wind, so that fogs are frequent and general conditions are cool and humid.

## II.  Plant communities

The vegetation of the park may be divided into four physio-graphic formations: (A) a relatively dry steppe, or coniferous savannah, on the eastern plateau; (B) a hydrophytic formation on the wet ledges of the western portion of the park; (C) a pioneer formation on the moraine of the Two-Ocean Glacier; (D) a forma-tion of the rock terraces north and south of the glacier. In describ-ing the content of the plant communities, there has been no attempt to make exhaustive lists of species, as this study was primarily



FIG. 2.—General view of eastern steppe formation, showing curved lines of conifers governed by buried ledges.

genetical rather than morphological. Chief prominence has been given to the dominant species, and to those regarded as of genetic importance. The seasonal aspect described is that of early and mid-summer, and for that reason relatively less attention could be given to grasses and composites. The nomenclature throughout is that of RYDBERG's Flora of the Rocky Mountains and adjacent plains.

### A.  EASTERN STEPPE FORMATION

East of the shallow central stream valley the ground is rolling and relatively dry, and is covered by a grass formation dotted with scattered clumps of evergreens (fig. 2). This steppe vegetation con-tains at least four distinct types of community, whose composition

is determined largely by ground conditions. These are: (1) communities of snowbank depressions; (2) communities of the open steppe; (3) xerophytic conifer clumps; (4) mesophytic conifer groves.

1. COMMUNITIES OF SNOWBANK DEPRESSIONS.—These depressions are usually long shallow troughs, which are filled with snow until late in June or the middle of July. In them the soil is gravelly, with considerable humus, and is fairly wet although not saturated. In the bottoms of the troughs the plants are chiefly grasses and sedges, of which *Poa alpina, Carex Tolmeii,* and *Juncoides parviflorum* are the most prominent, together with *Claytonia lanceolata.* On the sides of the depressions the *Claytonia* becomes scanty, and its place is taken by *Erythronium grandiflorum,* which grows profusely in these localities, and frequently forms almost pure colonies in close stand. Here, as elsewhere in the mountains, *Erythronium* comes up early, even pushing its way through the snow and blossoming before it has melted away. *Claytonia,* on the other hand, does not appear until the snow has completely left the ground. When the snow first melts, the grasses are dead and dark in color, but later they dominate the bottoms of the depressions, while the spring flowering plants become practically invisible.

2. COMMUNITIES OF OPEN STEPPE.—On the low ridges and level tracts between the snowbank depressions is found the characteristic steppe vegetation. This consists chiefly of grasses and sedges, with local colonies of *Phyllodoce empetriformis* and *P. glanduliflora, Salix petrophila,* and *Dasystephana calycosa.* There are also scattered individuals of many species of mountain plants, of which the most prominent are *Erythronium grandiflorum, Castilleja* sp., *Dodecatheon pauciflorum, Pedicularis bracteosa, Veronica Wormskjoldii, Sibbaldia procumbens, Pulsatilla occidentalis, Valeriana sitchensis, Pectianthia Breweri, Micranthes Lyalli, Hypericum Scouleri,* and at least one undetermined species each of *Potentilla, Geum, Antennaria, Arabis, Ranunculus,* and several composites. On the drier elevations the ground cover is scanty and open in stand, and the occasional bowlders and patches of bare rock have a scanty growth of crustose lichens.

3. ISOLATED CONIFER ASSOCIATIONS.—The conifer clumps of the east side contain a few trees each, varying from 4 to 20 feet in height,

surrounded by a narrow zone of shrubs and herbaceous plants. The
trees grow in close stand and form such a dense tangle that usually
there are no ground plants under them. The tree species are *Abies
lasiocarpa* and *Picea Engelmanni*, with an occasional pine (probably
*Pinus albicaulis*). The narrow shrub zone is made up mostly of
*Vaccinium membranaceum* and *V. scoparium*, with some *Dasiophora
fruticosa* and occasional specimens of the taller and more vigorous
steppe plants. These clumps grow on a dry substratum, in which the
soil is thin and sandy, and the underlying rock near the surface.
They are either round or oval in shape, or else elongated into long
strips or belts. The outlines of the clumps seem to be determined by
the topography of the underlying rock, as the trees grow only where
the rock is near the surface. Over long ledges of rock the clumps are
linear in shape, while the oval ones are found over isolated knobs or
hillocks of rock. The rock foundation of the northeast corner of the
plateau was originally rounded by erosion, so that the ledges have a
semicircular, concentric arrangement. These are covered by a thin
layer of soil, but the underground topography is clearly indicated by
the arrangement of the rows of conifers (fig. 2).

   4. MESOPHYTIC CONIFER GROVES.—On the north side of the
eastern plateau the ground slopes toward the gulch which borders it
on the north. Here the exposure is less extreme, and consequently
the trees are larger, the groves more numerous, and the undergrowth
assumes a more mesophytic character. The clumps of trees are close
together and in some places almost continuous, and the spaces be-
tween are occupied by thickets or small meadows. The trees and
shrubs are the same species as those found in the isolated conifer
clumps, but they are larger and show a better vegetative growth.
The ground plants include many of the steppe species, especially
*Castilleja* sp., *Erythronium grandiflorum*, *Pedicularis bracteosa*, and
*Dasystephana calycosa*. In addition are found such mesophytic
species as *Veratrum Escholtzianum*, *Thalictrum megacarpum*, *Viola
glabella*, *Heracleum lanatum*, and *Xerophyllum tenax*.

### B. WET LEDGE FORMATION

   West of the stream which bisects the park, environmental con-
ditions are very different. The ground rises in broad steps to the

slopes of Mt. Clements and Mt. Oberlin, and on these the snow lasts much longer, owing to the protection of the mountain peaks to the west (fig. 3). The soil is soggy with water, and there are very few stretches of the dry steppe which characterizes the eastern section. The drier portions resemble the snowbank depression of the east side, and the characteristic plants are *Erythronium grandiflorum*, *Claytonia lanceolata*, two species of *Phyllodoce*, and *Salix petrophila*. Most of the ground is covered by a formation resembling a wet Arctic tundra. The little streams and ponds are bordered by dense



FIG. 3.—Wet ledge formation on west side of park, below Two-Ocean Glacier

mats of hydrophytic mosses, with some of the more hydrophytic species of the east side and some species not found on the east. Among the steppe species are *Claytonia lanceolata*, *Pedicularis bracteosa*, *Veronica Wormskjoldii*, several saxifrages, and *Pulsatilla occidentalis*. Among those not found on the east side are *Kalmia microphylla*, *Anticlea elegans*, *Mimulus Langsdorfii*, *Pyrola secunda*, and *Tofieldia palustris*.

In one spot a little patch of Arctic muskeg contained *Sphagnum* and the rather rare heather, *Cassiope Mertensiana*. This is a close relative of *C. tetragona*, which is so characteristic of the Arctic barren lands that its woody stems often furnish the only fuel available for

Arctic travelers. The Rocky Mountain species is found only in isolated spots through the western mountains, and, so far as known, has not previously been reported in Glacier Park. The *Sphagnum* also has not previously been reported at this altitude in Glacier Park, although it is found in large bogs above the head of Lake McDonald on the west side of the divide. The only community of this type was found along the stream which flows from the northeast end of Two-Ocean Pond, but it seems likely that others could be found in this portion of Alpine Park. It is probable that its presence there may be explained by the cold wet conditions to be found below the sheltered snowbanks under the slope of Mt. Clements.

C. COMMUNITIES OF TWO-OCEAN GLACIER MORAINES

Two-Ocean Glacier is located at the base of the eastern cliff of Mt. Clements. Its general shape is that of a slender crescent with a long curving front, and a serrate collecting surface, with points extending up the side of the mountain. The moraines border the front in two rows, and are steep, disconnected ridges of dry, powdery clay with a large admixture of angular argillite. The inner moraines are the younger, and on them there is almost no plant life to be found. The few plants present occur as individuals widely separated from one another, and generally stunted in habit. The species include two or three grasses, of which *Poa alpina* seems to be the first to appear, followed by *Juncoides parviflorum* and *Carex Tolmeii*, together with *Papaver pygmaeum, Silene acaulis, Pentstemon ellipticus* and *P. virens, Epilobium anagallidifolium*, a chickweed, and two composites, probably *Arnica* and *Erigeron*.

On the next line of moraines the plants are found in small clumps about one foot apart, and include the species found on the first moraine with a few additional ones. Among these may be noted *Valeriana sitchensis*, a white *Phacelia*, an *Antennaria*, a dwarf *Taraxacum*, and at least one specimen of *Polystichum Lonchitis*.

D. ROCK TERRACE FORMATION

On either side of the Two-Ocean Glacier the moraines taper out on the wet ledge formation, but farther north and south are found rock terraces, which are formed by the outcropping of the strata in the bases of Mt. Oberlin on the north, and a spur of Mt. Reynolds on

the south. These strata dip slightly to the west, so that their outer
edges are higher than the inner, and are consequently dry, with a
shallow depression at the base of the terrace next above (fig. 4).
The vertical faces of the terraces range from 6 to 10 or 12 feet in
height, while their level surfaces are from 10 to 20 feet in width.
The outer edges generally carry a narrow belt of stunted evergreens
of the same species as those of the clumps on the steppe. The char-
acter of the vegetation of the shallow inner trough varies with the
amount of water collecting there. In very wet localities the com-



FIG. 4.—Rock terraces southeast of Two-Ocean Glacier

munities resemble those of the wetter portions of the main plateau.
Hydrophytic mosses, sedges, and grasses predominate, with *Gen-
tiana*, *Pedicularis*, saxifrages, the two species of *Phyllodoce*, and
occasionally *Claytonia* and *Pulsatilla*.

On the drier border between the trough and the conifer belt, and
also in the troughs which contain little water, the ground cover is
continuous, and resembles the vegetation of the dry steppe. The
vertical face of each terrace is usually dry, and xerophytic mosses,
crustose and foliose lichens, grasses, and such flowering plants as
rockcress, chickweeds, and one or two saxifrages grow in crevices
and pockets of the rock face.

When the water from above is abundant, it trickles down over the face of the terrace, and the vegetation is more luxuriant and mesophytic, or even hydrophytic. Mosses are abundant, with lichens and some ferns, and even liverworts. Among flowering plants are *Thalictrum megacarpum, Saxifraga* spp., *Viola glabella*, and local colonies of the rare *Romanzoffia sitchensis* and *Pinguicula vulgaris*. Farther from the glacier the terraces gradually disappear, and the vegetation takes on the form found regularly on valley walls and upper mountain sides.

### III.  Development of plant communities

A region occupied by retreating glaciers is one of the few spots on the earth's surface affording evidence as to the order of development of vegetation on clay as well as on bare rock. A comparative study of the communities in Alpine Park should furnish some information as to the composition and order of the successive communities which have appeared in these valleys and on the mountain sides.

A general survey of the various communities found in this region would indicate that the climax community, under present conditions, is the heavy conifer forest found in the river valleys of the east front. The forest of the McDonald Creek Valley is even more luxuriant, but it also contains many Pacific Coast species which are not found east of the divide on the crest of the Lewis Range. The exact relationship of these two forests cannot be stated definitely, until more is known of the general relationship of the Rocky Mountain and the Pacific conifer forests. For this reason, only the succession of the vegetation of the east slope will be considered here.

As one leaves the heavy forest on the valley floor and ascends the slopes, the trees are found to be more open in stand and more xerophytic in character. Thickets and mountain meadows appear in gaps in the forest, and they become more extensive as the timberline region is approached. The glacial moraines are usually found just below timberline, and are generally bordered by exposed rock terraces and ledges, which characterize the bases of the adjoining mountain.

In this region the progressive changes in vegetation which were vertical on the valley sides, now become horizontal. The trees take on timberline characteristics, and are stunted and scattered, until

they pass into meadow or steppe, and finally into the plantless areas of the youngest moraines. Reversing this order, it will be seen that the latest moraines represent the pioneer habitats of the succession, the rock ledges and upper slopes carry the intermediate stages, and the river valleys the climax stages. Two successions may be followed, one beginning on the mixed clay and gravel of the moraines, the other on the bare rock ledges or terraces.

## A. CLAY-GRAVEL SUCCESSION

1. XERARCH.—The pioneer stage in this succession is found in the youngest moraines of the Two-Ocean Glacier. A comparison of photographs taken in 1905, 1919, and 1923 indicates that all the glaciers of Glacier Park receded very little between the first two dates, but retreated rapidly between 1919 and 1923. Apparently the inner moraine was at least partly covered in 1919, and so the scanty vegetation found on it has come in since that date.

The first pioneer plants thus seem to appear on the dry moraines within a few years after the melting of the ice. The absolute pioneer is *Poa alpina*, and a few flowering plants such as *Papaver pygmaeum*, *Silene acaulis*, two species of *Pentstemon*, *Epilobium anagallidi-folium*, a chickweed, and two composites. The individual plants are widely separated, stunted in size, and grow very slowly. They increase rather slowly in size and in number of stands, as indicated by the conditions observed on the second moraines. So far no attempt has been made to set a date for the uncovering of this line of moraines, as little is known of the rate of recession of these glaciers previous to 1900. The rate of invasion by the plants of the secondary stages can be estimated only in a very general way.

On this outer line of moraines the grasses and sedges have increased in importance out of proportion to the other plants, and the composites also have increased in number and area covered. On the whole the vegetation is denser at the ends of the line nearest the flanking mountain slopes, and representatives of conifer species have begun to appear. It was in this part of the moraine that *Polystichum* was found. There are no conifers on the moraines of the Two-Ocean Glacier, but in similar situations on the moraines of other glaciers in Glacier Park small clumps of conifers are found on the

outer edges of the older moraines. These are usually tree species, chiefly alpine fir, Engelmann spruce, and whitebark pine, but *Juniperus sibirica* is frequently associated with them. They are all extremely stunted in habit, and are located either in slight depressions or on protected slopes of the moraine ridges. The clumps seem to increase in size very slowly, and do not show any tendency to cover the ground completely. From these facts it would appear that the plants of the secondary stages have migrated slowly from the communities already established on the adjoining mountain sides, rather than from the steppe below.

As the moraines are generally separated from the steppe by the wet ledge formation, it is difficult to determine the relationship between the two, but apparently the steppe represents a temporary climax on a dry clay-gravel substratum. The conifer groves on the steppe do not seem to be increasing in size, and as they are definitely connected with buried rock ledges, it would seem as if they early became established on these dry spots and have not been able to advance over the grass mat of the steppe. The fact that the groves are more mesophytic on the sheltered northern slope of the plateau, might indicate that under favorable conditions they increase in size and eventually cover the whole area.

2. HYDRARCH SUCCESSION ON CLAY-GRAVEL.—Glacial ponds show no traces of the earlier stages of the regular aquatic succession. These waters are clear and plantless from the center to the edge of the surrounding land, and the banks, although low, generally have vertical faces at the water's edge. The absence of aquatic plants is probably due to the short ice-free period and the very cold water. Even if the plants could grow in the cold water, the supply of seeds would be very scanty on account of the high gradient of the outflowing streams and the small numbers of aquatic birds which visit them from the valleys. In the small ponds and troughs and the narrow streams which flow from them, the pioneer plants seem to be hydrophytic mosses of the hypnum type, and rarely sphagnum. These form dense water soaked cushions, under which a sort of peat accumulates. In this wet substratum the first flowering plants to appear are usually *Saxifraga Lyalli* and *Pedicularis bracteosa*, followed by *Phyllodoce* spp., *Kalmia microphylla*, and the other species

already noted. The *Sphagnum-Cassiope* association is found only in
isolated patches, and there was no evidence to show whether or not
it constitutes a stage in the succession (fig. 5). Apparently it is con-
temporaneous with the hydrophytic communities with which it is
associated, but only a microscopic examination of the underlying
peat deposits could establish this point.

The progress of the development on the borders of these ponds
and streams is very slow, but the wet meadow or snowbank depres-
sion communities apparently invade the peat beds as the substratum
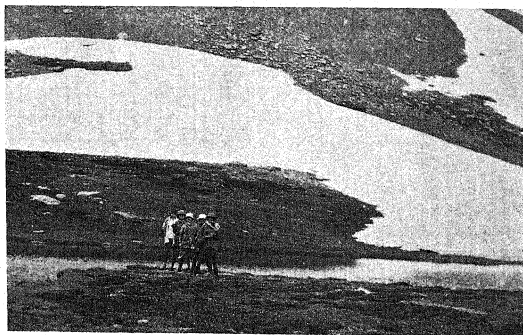


FIG. 5.—Two-Ocean Pond; *Sphagnum-Cassiope* association found a few hundred
yards to right of this pond.

becomes drier, and the open steppe formation would seem to be the
temporary climax of the hydrarch succession, under present climatic
conditions.

### B.  ROCK SUCCESSION

1. XERARCH.—The pioneer plants on bare rock surfaces are
crustose lichens, ranging in color from black and white to brilliant
yellows and reds. These are abundant on both vertical and horizon-
tal surfaces near the mountain summits, and they also occur fre-
quently at lower levels. This pioneer stage seems to continue for
long periods of time with very little change, as lichen covered rocks

are to be found even among conifer groves. This is probably due to
the small amount of dust present, and also to the washing effect of
the frequent rains. For these reasons soil does not accumulate
around the lichens, and plants of the later rock stages are unable to
get a foothold. Where advanced stages of vegetation are found on a
thin layer of soil over rock, the character of this soil makes it seem
probable that it was left there after the retreat of the ice, rather than
that it accumulated in connection with the development of vegeta-
tion. Where the rocks have cracks or pockets, crevice plants have
become established, and even stunted conifers may be found. Even
here, however, the development has not advanced on the rock, as
there is little extension of the vegetation beyond the limits of the
soil holding pockets.

2. HYDRARCH.—In rock troughs and shallow basins in which
water stands throughout the summer, development has usually
made some progress. The miniature pond will usually be bordered
by a zone of hydrophytic mosses, sometimes equalling or exceeding
in width the relict pool in the center. This moss carpet will be under-
lain by a layer of peat, the thickness of which will depend on the
topography of the rock and the distance from the center of the pool.
The deposit of peat will naturally become thinner toward the edges
of the original pool, except where local depressions in the rock held
secondary pools around the edges of the main body of water.

In the moss cushion growing above this peat, a few angiosperm
species have become established. These hydrophytic pioneers in-
clude *Saxifraga Lyalli, Pedicularis bracteosa*, and *Tofieldia palustris*,
but as the peat becomes drier, other members of the wet clay-gravel
meadow invade. As the rock ledges around these ponds generally
carried a clay-gravel deposit, a careful examination of the sub-
stratum is necessary to determine whether these secondary stages
have developed on peat or on morainic soil. Of course in cases where
the peaty character of the soil is established, these stages may be
regarded as truly belonging to a rock succession, but in a majority of
cases the development on peat appears not to have progressed very
far. These observations, taken with hints from other localities, make
it seem probable that the development of advanced stages of a cycle
is relatively rare on a bare rock substratum. The progress of suc-

cession on bare rock is so slow, that unless it can be demonstrated that a peat soil extends quite to bed rock, it seems probable that the later stages have developed over morainic material rather than over rock.

On vertical rock surfaces over which an abundance of water is always flowing, a hanging curtain of hydrophytic mosses may develop. This will reach considerable thickness on miniature ledges and in pockets, and here also the pioneer hydrophytic angiosperms of the horizontal moss carpet will be found.

## Summary

1. The area described is located near Logan Pass in Glacier Park, Montana, and consists of a level tract about one mile square between mountain peaks, at an average elevation of 7000 feet.

2. Four physiographic formations were found: a grassy steppe dotted with clumps of conifers, a wet Arctic meadow between low rock ledges, a glacial moraine formation, and a rock terrace formation.

3. The xerarch succession of these communities proceeds from pioneer stages on the glacial moraines, to grass steppe dotted with conifer clumps. The steppe seems to represent a temporary climax, with the conifers as a possible later stage under more favorable conditions.

4. The hydrarch succession develops in ponds left by the melting glacier, but does not proceed further than a wet meadow stage.

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# COMPARATIVE MORPHOLOGY OF CYTOPLASM AND CHROMATIN

CHARLES J. CHAMBERLAIN

(WITH PLATE XII AND THREE FIGURES)

A study of the eggs of various cycads, even with the technique of twenty years ago, indicated that they furnish unusually good material for an investigation of the structure of cytoplasm, just as the Cucurbitaceae furnish unusually good material for a study of sieve tubes; while a study of chromatin, especially in root tips, with graduate students, raised the question whether the structure of cytoplasm and chromatin may not be fundamentally identical. The accumulated impressions from studies with classes, together with the writer's own investigations, have prompted the assembling of the results and the drawing of some conclusions.

## Cytoplasm

The eggs of cycads are the largest in the plant kingdom, reaching a length of 6 mm., with a diameter of 2 mm. Although very small, compared with the eggs of the bird *Aepyornis*, which reach a length of 30 cm. with a diameter of 20 cm., or even when compared with the eggs of living birds, like the ostrich or emu, it may be doubtful whether these enormous eggs had nuclei as large as the egg nucleus of *Dioon edule*, which sometimes reaches a diameter of 500 $\mu$.

The cytoplasm of the cycad egg, or central cell as it is called, is extremely vacuolated before the mitosis which results in the formation of the ventral canal nucleus and the egg nucleus, because it increases immensely in volume without much increase in the mass of cytoplasm. Some of the vacuoles are so large that they can be seen with the naked eye, but as the egg approaches maturity, a nutritive jacket develops about it, the cytoplasm increases rapidly, and the vacuoles disappear, until at the time of fertilization not many vacuoles can be seen with a high power dry lens.

Most of the investigations upon the structure of cytoplasm have been made by zoölogists, who have formulated various theories, notably the framework theory of FROMMANN, the filament theory of FLEMMING, the granula theory of ALTMANN, and the foam theory of BÜTSCHLI. E. B. WILSON's investigations upon echinoderm eggs proved conclusively that cytoplasm is an emulsion, and gave such strong support to BÜTSCHLI's theory that it has become generally accepted. According to BÜTSCHLI, there is a groundwork consisting of an immense number of closed spaces filled with watery liquid. The diameter of the spaces is usually less than a micron. WILSON found larger spaces, up to 3–4 $\mu$ in diameter. STRASBURGER had described some small spaces, but also some much larger spaces, which BÜTSCHLI claimed did not represent the real structure of cytoplasm, although he admitted that STRASBURGER occasionally got glimpses of the real structure.

In dealing with paraffin sections, technicians know that it is easier to get good preparations from animal than from plant tissues. This is because the vacuoles, or alveolar spheres in animals are so small, usually only 1–3 $\mu$ in diameter, while in plants the vacuoles are usually much larger. In the sporogenous tissue of plants, in spermatogenous tissue, in embryos, in meristem, and also in the whole group of Cyanophyceae, where the vacuoles are as small as in animals, the technique is easy; but in embryo sacs, endosperm, and most plant cells, where the vacuoles are very large, the technique is difficult.

This study of the cycad egg endeavors to show that there is an unbroken series, from large vacuoles 100 $\mu$ or more in diameter, down to the smallest spaces demanded by the theory of BÜTSCHLI and WILSON, and that the largest and smallest spaces are of the same morphological nature. Then, in the study of the chromosomes of the root tip and microspore mother cell, I shall try to show that the structure of the chromatin is only such a modification of the structure of the cytoplasm as should be anticipated if the chromatin has been derived, phylogenetically, from the cytoplasm. This involves the supposition that the original living matter was not differentiated into chromatin and cytoplasm, that differentiation having come later.

A complete series of stages showing the condition of the cyto-
plasm during oogenesis of a cycad should begin with the archesporial
cell (if there is such a cell in cycads), or at least with the megaspore
mother cell; but, so far as I know, only three persons have ever
secured preparations of that megaspore mother cell. F. GRACE
SMITH finally found it in *Zamia floridana* by going to Miami where
the plant grows; TREUB found the megaspore stage in *Ceratozamia;*
and LANG found the same stage in *Stangeria*. The fact that cones
are covered in the bud during early stages in oogenesis makes it
practically impossible to secure material in greenhouses.

During these early stages any vacuole would be very small, for
the entire megaspore mother cell could not have one-fiftieth the
length of a mature egg. Stages in the development of the female
gametophyte show that the vacuoles increase rapidly in size until
the central cell reaches its maximum length of 3–6 mm.

Most of the illustrations are chosen from *Ceratozamia mexicana*,
beginning about fourteen weeks before fertilization. At that time the
central cell has the appearance shown in the photomicrograph (fig.
1), and the drawing of another section of the same cell (fig. 4). The
size of this cell is enormous, as compared with the surrounding cells,
which are about as large as the original archegonium initial; but
the increase in mass of cytoplasm has not kept pace with the in-
crease in the size of the central cell.

No attempt was made to determine the chemical nature of the
liquid in the vacuoles, but at this stage it is almost watery in con-
sistency, and the big cell is extremely turgid. If, in trimming ma-
terial for fixing, the blade comes too near the central cell, liquid
spurts out so violently that it can be felt at a distance of several
centimeters. This extreme turgidity continues throughout the
development of the central cell and egg, and even after fertilization,
until the mass of cytoplasm begins to be absorbed by the growing
embryo.

The largest vacuole shown in figs. 1 and 4 has a diameter of
150 μ, but the smaller vacuoles are of the same morphological nature.
Under greater magnification the cytoplasm between vacuoles, which
appeared to be nearly homogeneous under the lower power, shows a
series of vacuoles of various sizes down to the limit of vision (figs. 2,
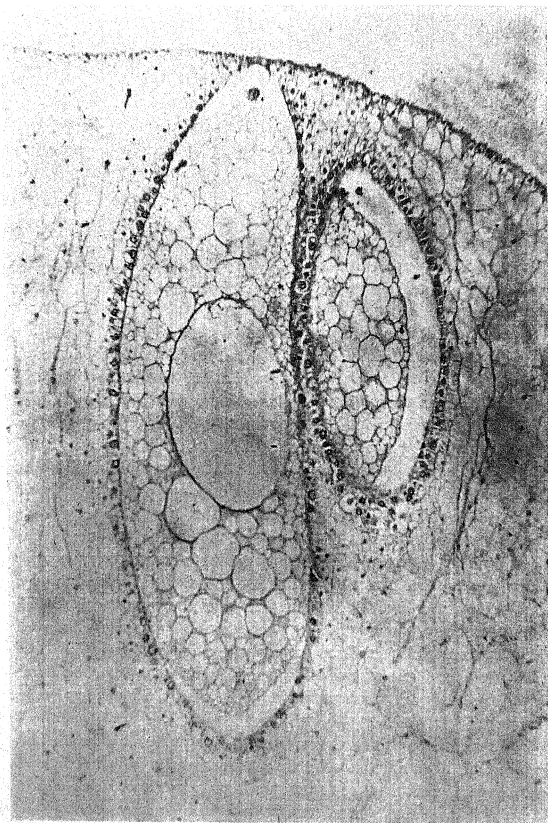
FIG. 1.—*Ceratozamia mexicana:* central cell about 14 weeks before fertilization; ×100.
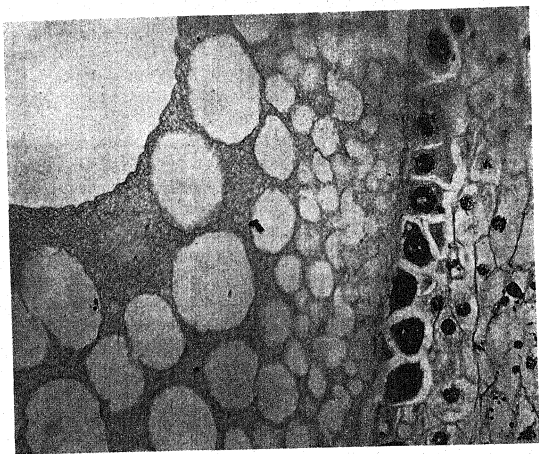
FIG. 2.—*Ceratozamia mexicana:* central cell 10 weeks before fertilization; ×847
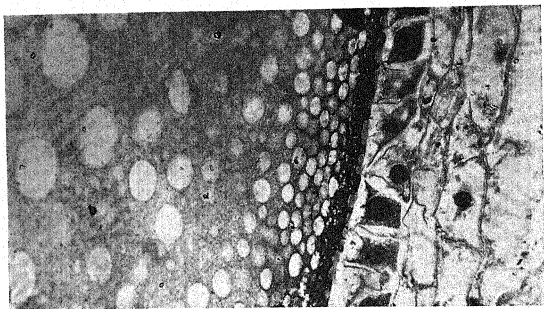


FIG. 3.—*Ceratozamia mexicana:* central cell 8 weeks before fertilization; ×613

5). With a Zeiss 2 mm. apochromatic objective, 1.4 N.A., and a
15× compensating ocular, vacuoles much less than 1 $\mu$ in diameter
can be recognized (fig. 5). It is probable that if a much higher
efficient magnification could be obtained, still smaller vacuoles
would become visible.

During these early stages in the growth of the central cell, it is
thought that the vacuoles are alike morphologically, and that their
contents are more or less uniform; but as the endosperm becomes
packed with starch and various materials, the central cell develops
its large haustoria and takes in substances of various kinds. Among
these are proteids, which often take a crystalloid form. All cycads
contain poison. It may be that some of the globules and irregularly
shaped masses constantly present in the endosperm during the later
stages of its development are the poisonous material, and it is pos-
sible that some of the vacuoles of the eggs in its later stages become
filled with the poison.

At a stage even earlier than that shown in fig. 6, about two
months before fertilization, globules which stain deeply with safra-
nin and iron haematoxylin appear in the central cell. Morphologically
they are not different from the substances which filled the other
vacuoles shown in this figure, but which were dissolved in the
preparation for microscopic examination. During the two months
which elapse between this stage and fertilization, great quantities
of material are taken into the central cell. So long as the walls of
the jacket cells remain intact on the side next to the central cell, any
material must pass by osmosis (fig. 6); but as the time for fertiliza-
tion approaches, these walls are broken by the haustoria, and
material in masses can pass from the jacket cells to the egg as readily
as from one part of the jacket cell to another (fig. 7). Material
filling some of the larger vacuoles in the peripheral portion of the
egg breaks into large numbers of small globules, as shown in fig. 7,
occupying, at first, the position of the large vacuole, and then be-
coming scattered throughout the egg. The visible vacuoles become
smaller and smaller, while the quantity of apparently homogeneous
cytoplasm increases.

At the time of fertilization, which occurs a few days after the
division of the nucleus of the central cell, giving rise to the egg

nucleus and the ventral canal nucleus, the vacuoles are so small that only a few of them can be seen with a low power dry lens. At the instant of fertilization, cytoplasm breaking out from the egg between the two neck cells shows elongated vacuoles, and gives a definite impression of streaming (fig. 8). As it comes to rest just outside the neck cells, however, the vacuoles are much smaller, and at the periphery of the mass which flowed out, the structure is apparently homogeneous, with no recognizable vacuoles.

In *Stangeria*, at the time of fertilization, the egg contains innumerable small vacuoles. The immense size of the egg nucleus, as compared with the vacuoles, is seen in fig. 10, in which the nuclear membrane (*m*) shows scarcely any curvature. As the free nuclear period in the development of the embryo advances, starch appears in its periphery, probably brought in bodily from the jacket walls through the haustoria.

Until it completes the free nuclear stage, the embryo receives nutrition rather uniformly from all the surrounding cells; but as the cellular stage begins, the embryo breaks through the bottom of the egg, and receives more and more nutrition from that region, while the jacket ceases to furnish material. Soon all the material in the upper part of the egg is absorbed, and its original contour is maintained only by the very thick egg membrane.

Some of the later stages in the cytoplasm of the egg were not studied very carefully, but the structures are certainly developed from the vacuolated condition already described. Shortly before fertilization, it is common to find in the egg considerable areas which have a distinctly thready or reticulate structure. The globules and small granules which become increasingly abundant as fertilization approaches, I regard as contents of vacuoles, of a different character from the contents dissolved out from the rest of the vacuoles.

### Chromatin

So far as the actual structure of the chromatin is concerned there is nothing new to add. I simply affirm my belief that the chromatin in plants is a vacuolated substance like the cytoplasm, and that in the plants studied there are no such structures as chromomeres upon a linin ribbon. For many years our classes in cytology have

studied mitosis in various root tips, especially those of *Tradescantia virginica*, *Vicia Faba*, and *Trillium grandiflorum*. The vacuolated condition during telophase, resting condition, and early prophase seems evident. The figures are drawn to the same scale as figs. 5–11, from sections 3 μ thick, stained in iron-alum haematoxylin. A modification of Flemming's weaker solution, with the osmic acid still weaker than in that formula, was used for fixing.

The chromatin from anaphase to late prophase of the next mitosis is vacuolated. The structure is the same as that of the cytoplasm, except that the vacuoles are much smaller, and, since the chromosomes are elongated, are arranged more or less in rows. In places where there is only one row of rather uniform vacuoles, the resemblance to chromomeres on a linin thread is quite pronounced (figs. 12, 13, and right hand part of 14). In the great majority of cases, like the left part of fig. 14 and figs. 15–19, the vacuolization is evident.

### Theoretical considerations

It is hardly probable that the original living matter had the familiar complex mitotic arrangement for division. In spite of the fact that many lines of evolution show progressive simplification rather than increasing complexity, it is more probable that the original living matter was comparatively simple and that the differentiation into cytoplasm and chromatin came later. A comparative study of the Cyanophyceae might show stages in such a differentiation.

Definite chromomeres upon a linin ribbon afford a convenient resting place for genes, and facilitate philosophical speculation, especially if a theory demands a rigid serial arrangement of hereditary characters in the spirem. In a vacuolized chromosome there might be some serial arrangement, but it could not be so definite. On the other hand, if the vacuolization theory is correct, as it seems for all the plants I have studied, the chromosomes of early telophase would not be absolutely the same when they reach the next mitosis. Parts of the slender threads might be drawn back into the chromosomes from which they were drawn out; or they might break in such way as to go to the contiguous chromosome, and thus cause more or less variation. We are not dealing with theories, however. It

seems apparent that both cytoplasm and chromatin are vacuolated and of essentially the same structure. We should not claim that such structures as linin and chromomeres do not occur in some animals; but I feel certain that they do not occur in the plants studied, and I predict that theories which cannot be reconciled with a vacuolated structure of the chromosome will have to be abandoned.

UNIVERSITY OF CHICAGO

## EXPLANATION OF PLATE XII

The photomicrographs, text figs. 1–3, were made by Mr. C. Y. CHANG. Fig. 4 was drawn with a 16 mm. objective and a 6× ocular; figs. 5–19 were drawn with a Zeiss 2 mm. apochromatic objective, 1.4 N.A., and compensating ocular 15×; and all figures, except the photomicrographs, were reduced to ⅝ the original size. Fig. 4 is magnified 62 diameters; figs. 5–19 are magnified 1250 diameters.

FIG. 4.—*Ceratozamia mexicana:* central cell 14 weeks before fertilization, showing vacuoles of various sizes.

FIG. 5.—*Ceratozamia mexicana:* small portion of cytoplasm of central cell at stage shown in fig. 4; diagram 5a shows position of piece selected for the drawing.

FIG. 6.—*Ceratozamia mexicana:* portion of central cell 8 weeks before fertilization, showing thick egg membrane (shaded) and vacuoles of various sizes; parts of three large jacket cells shown at left.

FIG. 7.—*Dioon edule:* portion of egg about the time of fertilization, showing globules which break into groups of smaller globules as they penetrate farther into the egg.

FIG. 8.—*Ceratozamia mexicana:* cytoplasm streaming out between neck cells at fertilization.

FIG. 9.—*Ceratozamia mexicana:* cytoplasm just outside neck cells after escaping from egg.

FIG. 10.—*Stangeria paradoxa:* small vacuoles in cytoplasm at time of fertilization; right hand part of figure shows small portion of egg nucleus; *m*, nuclear membrane.

FIG. 11.—*Stangeria paradoxa:* small portion of embryo in 32-nucleate stage; groups of starch grains (*s*) shown near periphery; small portion of one of the 32 nuclei shown at right.

FIG. 12.—*Trillium grandiflorum:* portion of chromosome in telophase of heterotypic mitosis in pollen mother cell.

FIG. 13.—*Trillium grandiflorum:* somewhat earlier stage than that shown in fig. 12.

FIG. 14.—*Trillium grandiflorum:* telophase of homotypic mitosis in pollen mother cell.

FIG. 15.—*Trillium grandiflorum:* slightly earlier telophase of homotypic mitosis than that shown in fig. 14.

FIG. 16.—*Trillium grandiflorum:* chromosome in telophase of heterotypic mitosis in pollen mother cell.

FIG. 17.—*Tradescantia virginica:* portion of chromosome in prophase in root tip.

FIG. 18.—*Tradescantia virginica:* portion of spirem in prophase in root tip.

FIG. 19.—*Tradescantia virginica:* portion of spirem in slightly later prophase in root tip than that shown in fig. 18.

PLATE XII



4

5

5ᵃ

8

9

6

10

m

7

11

s

s
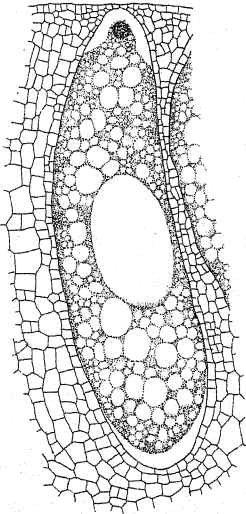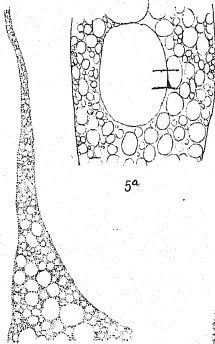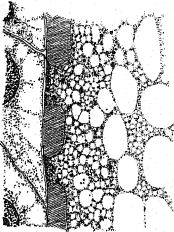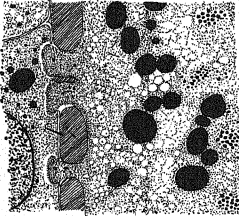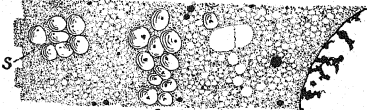
12    13    14    15    16    17    18    19
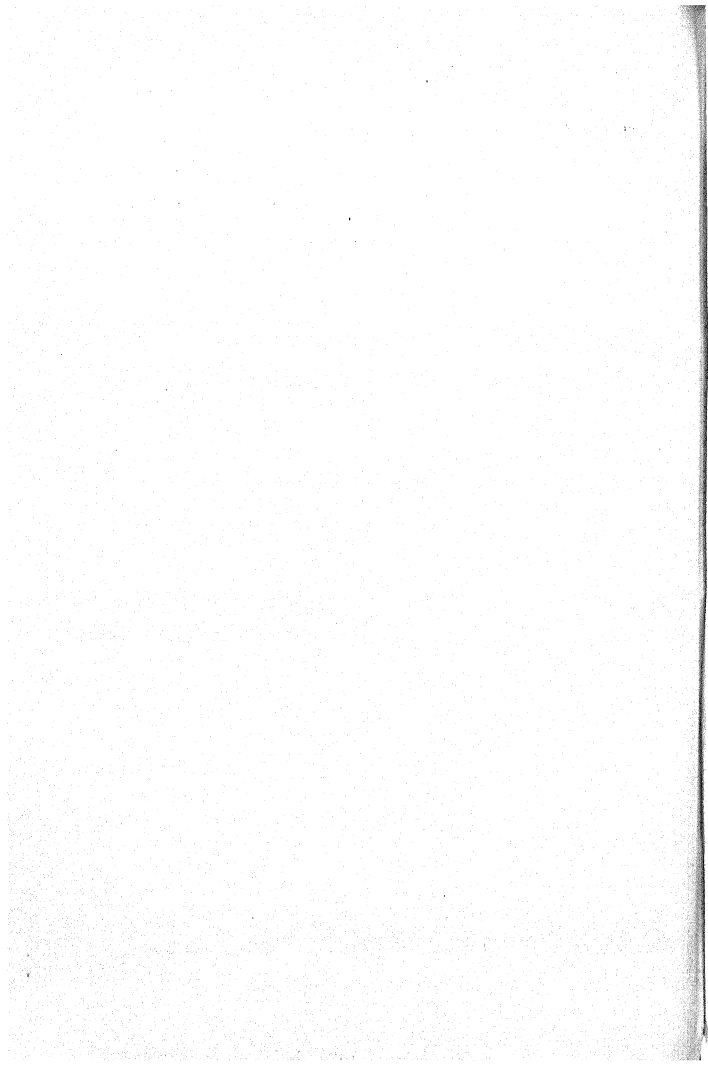
# VEGETATION OF NORTH GREENLAND

## C. H. OSTENFELD

### I

As Greenland extends from 60°N. to about 83.5°N., the condi-
tions for vegetative life are very different in its southern subarctic
part, where there are copses of birch (*Betula pubescens*), and in the
most northern portion, which supports the most northern vegetation.
The present paper deals only with the vegetation of North Green-
land, that is, Greenland north of 76°N. The botanical knowledge of
this part of Greenland has increased greatly during the last two
decades, due partly to American explorations, but mostly to the
information obtained by Danish expeditions.

Usually Arctic expeditions have included no trained botanists,
but one Danish expedition, the Second Thule Expedition under com-
mand of KNUD RASMUSSEN in 1916–1918, was an exception. Dr.
THORULD WULFF, a well known Swedish botanist, accompanied this
expedition, which explored the most northern coast of Greenland
facing the polar sea. Dr. WULFF succumbed to the hardships of the
expedition as land was reached, after having traversed the inland
ice just south of the Humboldt Glacier. He left a small collection of
plants, made with wonderful energy under the hardest conditions,
and also a diary containing many botanical and meteorological
observations.

It has been the writer's privilege to identify the higher plants
of this collection and to make use of his diary. The results are em-
bodied in a recently published paper,[1] of which the first part of the
present article is to some extent a résumé. It is rather surprising
that this flora contains not less than seventy species of higher plants,
that is, flowering plants and vascular cryptogams, in spite of the
poor conditions under which they live. If we examine the main
factors of importance to plant life, we find the following points of
interest.

1. In the short portion of the year during which plants are able

to grow in these high arctic latitudes (82°–83°N.), the sun is constantly above the horizon and consequently the plants are able to assimilate without interruptions due to darkness. From WULFF's observations it appears that during the three months, May to July, when he worked on the north coast, there was much sunshine; he has nearly one hundred meteorological observations for each month and about half of them report sunshine, while there are very few notes about fog or mist (hardly 10 per cent of the observations).

2. The temperature is not favorable. According to WULFF's observations, the air temperature during the three months shows that in May it never reached the freezing point, in June the mean temperature was about zero (C.), and not until July was there a

TABLE I

TEMPERATURES (C.) FROM WULFF'S OBSERVATIONS

| Month | No. of observations | Mean |
|-------|---------------------|------|
| May....................... | 93 | −8.7 |
| June...................... | 110 | −0.1 |
| July...................... | 100 | +2.65 |

mean temperature above the freezing point (table I). There are observations only for the first three days of August, but if we take observations from other arctic places into consideration, we may conclude that the mean temperature is not much above zero, most probably near to it (table II). This means that the plants have only one month in the year in which they really grow, taking for granted that the flowering plants are not able to grow at temperatures below freezing. It must be borne in mind, however, that the plants absorb heat from direct insolation, and thereby get temperatures decidedly above that of the air. WULFF made several experiments to show this. He found such differences as the following: air temperature (May 19) −11.8°C., while the temperature in a tuft of moss was 9.2°C.; air temperature (June 20) −5°C., and that of a flowering tuft of *Saxifraga* 21.1°C. This of course is of importance for the plants, but if we compare his observations with others of the same kind from arctic regions of lower latitude, we find that the difference between the air temperature and that of plant tufts is less than we should expect.

3. The precipitation according to WULFF is rather inconsiderable in the summer, and most of it comes as snow, except in July. The air is very dry in the first part of the summer, and the evaporation in the uninterrupted sunshine is rapid. The snow evaporates so quickly that it disappears without making the ground wet. The plants, therefore, are liable to suffer from lack of available water, in spite of the apparent abundance of snow and water. The frozen soil intensifies this condition, and not before the rains and snow melting of late summer do plants get sufficient water.

TABLE II

MEAN TEMPERATURES (C) IN SOME HIGH ARCTIC REGIONS

| Region | Latitude N. | May | June | July | August | September |
|--------|:-----------:|:---:|:----:|:----:|:------:|:---------:|
| Frans Joseph Land (Cape Flora)................ | 79°56′ | −8.5 | −9.4 | 1.3 | 0.2 | −4.0 |
| Danmark Harbour, Northeast Greenland......... | 76°46′ | −8.2 | −1.1 | 3.3 | 2.3 | −4.4 |
| Fort Conger, Ellesmere Land................. | 81°44′ | −8.1 | 0.6 | 2.7 | 0.8 | −11.7 |
| North coast of Greenland.. | 82°83′ | −8.7 | −0.1 | 2.65 | ? | ? |

4. Air movements seem rather favorable. Only ten out of WULFF's three hundred records indicate a force of wind over 5 of the Beaufort scale, and one-half of his observations report no winds. At other seasons surely strong winds are much more common, but then most of the vegetation is covered by snow.

5. This snow covering is an important factor for the protection of plant life in these regions, and not until the middle of June does the melting reach any considerable extent, and at the end of July the snow begins to reappear. Thus the ground is bare only between one and two months, and in many places the snow melts off only during the most favorable years.

6. There are few data regarding the soil. Most of the surface soil of the north coast is loose, coming from a substratum of Devonian sandstone. The rapid disintegration forms a loose surface, consisting of stones and gravel, with finer elements (clay) between. In some places the melting snow and the streams produce a clayey soil, in others the stones prevail. The so-called "polygon-field" is very common on horizontal surfaces, consisting of a network of

fissures formed by the drying up and cracking of the soil. The vegetation is usually confined to the fissures, while the surface itself is bare. Another kind of soil peculiar to arctic and high alpine regions is that due to solifluction (or slumping), occurring on sloping ground during the melting of the snow when the surface soil attains a consistency resembling porridge, while the subsoil remains frozen. A slow motion is set up in the surface soil, due partly to the slope and partly to the steadily increasing water supply. This movement is comparable with that of a lava, and by it small stones are arranged in curved lines at right angles to the direction of flow. Such a phenomenon, of course, is quite unfavorable to plant growth, and is common on the Greenland north coast.

This summary of the factors governing plant life shows how difficult the conditions are for the plants. The vegetation is always very low and close to the ground. Usually the individuals stand scattered, and only on specially favorable places do continuous mats of flowering plants, mosses, and lichens occur. All the species of higher plants are perennial, as the short time of growth does not allow annuals to exist. They develop either as herbs or as dwarf shrubs, classed according to RAUNKIAER's biological types as hemicryptophytes and chamaephytes, the latter being well represented and characterizing arctic as compared with temperate vegetation.[2] Growth during the short season of course is very limited. A decumbent form of the arctic willow, *Salix arctica*, reaches the maximum length, and the thickest specimen with a diameter of hardly 2 cm. showed about fifty annual rings. A special feature is the rapid development of leaves and flowers, since most species flower and ripen their fruit in the course of one month, although doubtless many species do not develop ripe seeds every year.

There is but little insect life in these high arctic regions, hence the majority of plants must rely on self or wind pollination. A number of flowers are insect pollinated, however, and WULFF observed different kinds of flies, a butterfly, and some moths visiting flowers.

In his diary WULFF says that the so-called "Fjaeldmark" (WARMING) or fell-field is the only plant formation present, but his

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[2] RAUNKIAER, Der arktiske og antarktiske Chamaefytklima. Biolog. Studier tilegn. Eug. Warming. Köbenhavn. 1911.

definition of this term is rather vague. WULFF's opinion seems to be explained partly by the fact that his note was written early in the season, when the snow cover was only partly removed, and only the most hardy plants, those of the fell-field, exposed. If "fell-field" be defined as an arctic (and alpine) plant formation where the individual plants are so scattered that there is bare soil between them, and consequently no room competition, then it is doubtless the dominant formation in North Greenland.

Besides this fell-field, other formations are found on especially favored places, but without exception they are of very limited extent. On a few places manured by the excrements of mammals and birds, herbaceous mats with dense vegetation exist, with *Alopecurus alpinus*, *Puccinellia angustata*, and *Poa* spp. as dominant species.

Another formation is found in the many small bogs. The characteristic species of this formation are *Eriophora*, *Arctogrostis*, *Juncus biglumis*, *Saxifraga stellaris comosa*, *Pleuropogon sabinei*, *Carex aquatilis stans*, *Eutrema Edwardsii*, etc. The shore vegetation is very poorly developed. *Puccinellia phyryganodes* and *Cochlearia officinalis groenlandica* are the only real shore species. No continuous heath is present. The heath dwarf shrubs (*Cassiope tetragona* and *Dryas integrifolia*) occur only as scattered individuals.

## II

Following this short survey of the plant life of the north coast of Greenland, it seems appropriate to make a few remarks on the plant geography of Greenland north of 76°N., confining them to higher plants.

At present we know with certainty 125 species from this region, all confined to the coastal region, as the inner part of the country is covered by inland ice. Of these 125 species, most are of wide distribution in arctic regions: 85 species (or 68 per cent) are circumpolar, and 31 (or 24.8 per cent) are probably of western origin, as they are more or less common in arctic and subarctic North America, while most of them are absent from arctic Europe, although fourteen are found on the arctic islands north of Europe (Spitzbergen and Nova Zembla). Only six species are of eastern origin, that is, absent from arctic North America. The remaining three species are said to be confined to Greenland, but as two of them (*Braya Thorild-Wulffii*

and *Taraxacum arctogenum*) are recently discovered species closely allied to other better known species which are widely distributed in arctic countries, it is probable that they may yet be found in arctic North America. The third, *Lychnis (Melandryum) triflora*, is a well defined species which is common over a large part of Greenland. It would be strange if this species did not occur elsewhere in arctic America, and quite recently I have found that a plant from the delta of the MacKenzie River, *L. Dawsonii*, is a form of *L. triflora*. Altogether it seems that the three endemic species really ought to be referred to the group of western origin, as it is also less probable that a recent flora like that of North Greenland had any endemic species.

In the writer's opinion most of the arctic species are older than the ice age, which accounts for their presence both in North America and Eurasia. During preglacial times they occupied the polar regions but were forced southward by the advancing ice sheet, reaching much lower latitudes in both hemispheres. When the climate moderated and the ice retreated, the plants returned northward and reached the area occupied at present. It is quite possible that some of them may have survived the ice age in the arctic, but hardly as far north as North Greenland. Admitting this, it seems safe to say that the main part of the Greenland flora must have immigrated from other countries, and for North Greenland the only probable route was from Ellesmere Land, crossing the narrow Smith Sound to Inglefield Land on the Greenland coast at about 79°N. This immigration must have been easier during the postglacial epoch, when the climate was somewhat warmer than at present, and several species which now are found in Greenland only south of 76°N. may have come over at that time.

The same immigration route as that of the western species is probable for all the circumpolar species which did not survive in Greenland itself, as it is more natural to suppose that they immigrated to Greenland from America rather than from Europe. This means that, so far as North Greenland is concerned, only 6 of the 125 species reached there from arctic Europe. Thus the flora of North Greenland is almost entirely American from the phytogeographic point of view.

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# TERMINOLOGY OF THE UREDINALES[1]

### J. C. ARTHUR

As the knowledge of the rusts developed to the stage where more than one spore-form was recognized as belonging to a species, it became advantageous to employ generalized terms. For this reason the term teleutospore met with ready acceptance. It signified the last spore-form in the series, the sorus in which such spores were formed being called a teleutosorus. The serial position of other kinds of spores was not so readily apparent. Consequently no important attempt was made to extend this method of naming the several classes of spores, but instead, generic names were brought into use, such as uredospore and aecidiospore. It required no explanation that spores from an *Aecidium* were to be called aecidiospores, but what about spores from a *Peridermium* or a *Caeoma?* Logically they should be called respectively peridermiospores and caeomospores. In this way there came into use terms to cover nearly as many kinds of spores as there were generic names recognized, even going so far as to infringe upon the domain of the teleutospore with the term pucciniospore.

The reaction to this multifarious terminology arose from the recognition that many apparently diverse structures possessed a general similarity to the best known forms of *Aecidium*, both in form and function, and so the term aecidiospore was often made to cover nearly all catenulate spores borne in a peridium. When the peridium was absent they were usually called caeomospores; and stylosporic forms with or without a peridium were generally called uredospores. With this reduction of terms came the growing impression that the first two terms covered a primary form of spore, and that uredospores were a secondary form, sometimes considered as essentially conidia, leaving the term teleutospore to designate a third or final form.

This terminology worked fairly well with many of the most common and familiar genera, but had to be stretched to apply to the catenulate, verrucose spores without a peridium in the genus *Coleosporium*, and served very poorly in less familiar genera, such as *Kuehneola*, *Pucciniosira*, etc.

Thus the matter stood when in 1905 the writer[2] proposed new terms to replace a multiplicity of old terms, and more clearly to bring out homologies in the different stages in the life histories of the rusts. Instead of the prevailing terms which were mostly derived from generic names, the four terms: pycnium (from πυκνός, solid or compact), aecium (from αἰκία, an injury), uredinium (from *uredo*, a blight or blast), and telium (from τέλειος or τέλεος, complete or perfect), were designed to cover the different sorts of sori in every full spored, long cycle rust. The names of the spores were derived in all cases from the sori bearing them, without regard to form, surface markings, or accessories, and were respectively pycniospores, aeciospores, urediniospores, and teliospores. That these terms have proved serviceable is evident from the increasing number of mycologists who employ them.

It may be that the reason why others do not use the new terms, aside from natural inertia or undue conservatism, is the belief held by some that the terms spermogonium, aecidium, uredosorus, and teliosorus have come to have such definite application and to convey such clear concepts that there is no need of a new set of terms. In fact, to them a new set of terms really makes a difficult situation still more confusing. Even if we grant that such is the case, which is much to be doubted, yet the cumbersome form of the old names and their lack of uniformity fail to commend them.

In order to show what concepts underlie the new terminology, as well as its application, the following explanation is given. First let it be borne in mind that the new terms do not merely replace the older ones, but that they stand upon a distinctly different basis. In the first place, the terms primarily apply to the sorus, and not, as with the old terms, chiefly to the spores and their accessories. While this was the intent when the terms were first announced, yet only

[2] ARTHUR, J. C., Terminology of the spore-structures in the Uredinales. BOT. GAZ. **39**:219. 1905.

within a few years have facts been available to make possible a full demonstration of the correctness of this position. Very little was known regarding the origin and intimate structure of the several forms of sori in 1905, most of the knowledge being derived from the studies of SAPPIN-TROUFFY, BLACKMAN, and CHRISTMAN, who were chiefly interested in the behavior of the nucleus. The work of a gradually increasing number of cytologists during the intervening two decades now justifies the statement that the four kinds of sori are built upon essentially the same plan. In brief, each sorus arises from a primordium, which differentiates a hymenial layer that produces spores from its upper surface. This is equally true of a pycnium or a telium, of an aecium, whether in the form of the simplest *Caeoma* or the most complex *Aecidium*, and of a uredinium, whether conidial in appearance or provided with a peridium and catenulate spores. In each of the four kinds of sori there may be more or less highly developed accessories, such as pseudoparenchyma, buffer cells, paraphyses, peridium, etc., partly evanescent and partly permanent. This essential similarity in the fundamental structure of the four kinds of sori is reflected in the similarity of the new names applied to them.

All rusts, whatever their appearance or diversity of spore-forms, pass through a two-phase life cycle: the gametophytic phase with usually uninucleate mycelium, and the sporophytic phase with usually binucleate mycelium. Every species of rust having the ancestral, long cycle development without exception terminates the first or gametophytic phase with a sorus, the aecium, in which cell fusion takes place, giving rise to binucleate spores, while the second or sporophytic phase is terminated by another sorus, the telium, in which the two nuclei of the spores, which have descended from the aecium, are at maturity fused into one. The first, or gametophytic phase, bears another form of sorus, the pycnium, if not lost in retrograde development, which in the dim ancestral uredinalean past may have been a male organ of reproduction, but now is functionless. It is uninucleate throughout. The second, or sporophytic phase, bears still another form of sorus, if not lost in retrograde development, the uredinium, which is of a repeating or conidial nature. It is binucleate throughout. To recapitulate: every long cycle rust, without excep-

tion, terminates its first phase of growth with an aecium, and its second phase with a telium, and to the first phase may or may not be added a pycnium, while to the second phase may or may not be added a uredinium. There are no exceptions to this general statement, except such as are due to extreme simplification or are to be considered pathogenic or abnormal.

When the two essential kinds of sori to be found in every long cycle rust, the aecium and telium, are telescoped into one, a short cycle rust is produced, with the sporophytic mycelium omitted. In the short cycle rust the first, or gametophytic phase, ends as it does in long cycle forms with fusion of cells, while the second or sporophytic phase, which is very short, ends when the two nuclei of the resulting spores fuse as these spores reach maturity. In consideration of this combined action the sorus may be called an aeciotelium. Sometimes there are pycnia with a short cycle rust, but never uredinia.

The two preceding paragraphs have been devoted to a brief elementary statement of the course of development among the rusts. All rusts of every sort are thus seen to have a two-phase development, whether long cycle with two forms of mycelium, and two to four forms of sori, in which aecia and telia are always present, or short cycle with one form of mycelium, and one or two forms of sori, aeciotelia always being present. This simple statement should be born in mind in trying to determine the application of the new terminology.

In 1905, in the preface of an important paper, HARPER[3] stated the grounds on which comparative morphology and the use of terms should proceed, as follows:

Great confusion has arisen in the morphology of the fungi and algae from allowing considerations of functional equivalence or difference to mingle with and modify the conceptions of what should be a strictly phylogenetic morphology. If there can be agreement that the various developmental stages and fruit forms of the fungi and algae shall be classified and named in accordance with what can be determined as to their phylogeny, a number of disputed questions will disappear.

[3] HARPER, R. A., Sexual reproduction and the organization of the nucleus in certain mildews. Carnegie Inst. Wash. Publ. no. 37. 1905.

Two decades have passed since these statements were made, and before me lies a recent letter from HARPER, from which I have the privilege to quote, in which he says:

It is the great outstanding achievement which revolutionized comparative morphology that we have come to realize that structures should be named not according to their function, but according to their morphological and phylogenetic connections.

Probably no one is likely to dispute this reiterated statement of a fundamental doctrine of the phylogenetic basis of modern morphology, certainly not the writer. The difficulty arises in the application.

The present state of knowledge indicates that in the rusts there are four kinds of sori, arising at successive intervals in the life cycle of every species having the ancestral, full spored cycle of development, all possessing a certain unity of structure, but differing in histological details, and each having its independent phylogenetic history. When a retrograde change has taken place in the historical development of a species, one or more forms of sori may be omitted from the cycle, going so far as to leave only aecia and telia in long cycle species and aeciotelia in short cycle species. Beside the definite consecutive positions in the life history held by the four sorts of sori, each has its distinctive fundamental structure, determined by the nuclear behavior. There is no gross structural character that belongs to one kind of sorus rather than to another, although certain forms are met with so frequently as to seem characteristic. The true nature of a sorus depends upon its phylogenetic position in the individual cycle and upon its nuclear structure.

It would be illuminating to take up a number of representative species and contrast the application of the old and new terms, but it seems scarcely necessary. If the emphasis in thinking of the rusts is placed upon the mycelial body and the fruit forms or sori which it bears in unvarying order, instead of upon the spore forms and their accessories, there should be no difficulty in apprehending the simplicity of the new terms and their suitableness for every possible situation, whatever bewildering likeness the spores and their accessories may suggest.

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# BRIEFER ARTICLES

## THE MITOGENETIC RAYS

### (WITH ONE FIGURE)

In a recent paper[1] I reported on experiments which have demonstrated conclusively the possibility of the induction of mitoses in onion tips from other similar root tips over a distance up to 2 mm. If a root tip, occupying a horizontal position, be placed at the side surface of another vertical root tip for about three hours, a narrow median area of the "exposed" side of the latter, measuring approximately 70 $\mu$ in width, shows a con-
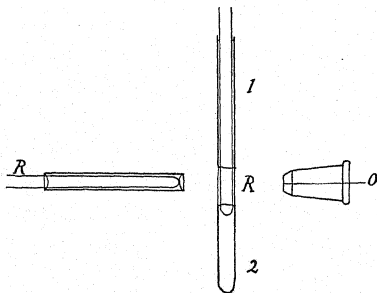
FIG. 1.—Arrangement of experiment with induction through air; both roots (R) placed in capillary tubes filled with water; vertical tube consists of two parts (1 and 2) between which root is covered only by a capillary water layer and is exposed to induction; O, horizontal microscope for controlling exactitude of position and growth of roots.

siderable increase (50 per cent and more) in the number of mitoses in comparison with the opposite side. Previous investigations, covering a period of many years, have shown that in "unexposed" root tips the difference in the number of mitoses on the "right" and "left" side in longitudinal sections exceptionally reaches 20 per cent, and alternates regularly.

The present paper reports other new facts concerning this peculiar

[1] Archiv. Mikr. Anat. Entwick. Roux. vol. 100. 1923.

mitogenetic factor, confirming the existence of a specific "mitogenetic" radiation, and giving some idea of its nature. The chief conclusions of the investigations, done with the collaboration of W. RAWIN, N. GURWITCH, and L. GURWITCH, are as follows.

1. Heteroinduction from a *Helianthus* root tip to an onion root is possible (RAWIN).

2. The induction is effected through water and through air as well (fig. 1).

3. The induction is effected, without visible decrease of intensity, over distances up to 38 mm. (maximal tested distance).

4. The interposition of a glass layer (cover slip) reduces, but does not totally suspend the effect of the induction. A glass plate of about 30 $\mu$ thickness seems not to reduce the action noticeably.

5. The interposition of a thin layer of plant tissue (onion skin, which can easily be peeled from the external surface of the layers of the bulb),

TABLE I

The action of induction is expressed in percentage of predominance of the inducted side in comparison with the non-inducted side:

| Induction | Percentage |
|---|---|
| 1. Simple, onion root tip on onion root tip.........2, (20, 23, 60, 27, 50, 26), 0[2] | |
| 2. Through onion skin | |
| 20, 26, 16, 25, 18, 20, 26, 15, 18, 10, 20, 26, 24, 30, 25, 27, 29, 24, 15 | |
| 3. Through a 30 $\mu$ wide slit ............................4, 0, (50, 24), 6, 2 | |

which is highly and regularly permeable to light rays, causes a considerable dispersion of the ray beam of the mitogenetic factor (table I). Whereas normally only 6–7 central sections show the effect of induction, under the conditions mentioned this is found in approximately twenty sections of 10 $\mu$, of course with a correspondingly decreased intensity. In view of the fact that a medium, transparent for light rays, causes a kind of dispersion of the mitogenetic factor, it seems probable that, if this factor is radiant energy, its rays must have shorter waves than light rays. It was found also that crystallinic quartz is completely transparent for mitogenetic rays, but that a thin film of gelatine will completely absorb them. This fact indicates that the wave length of the rays is in the neighborhood of 2000 Angström. Induction upon onion roots occurs also from embryonic animal tissues (small pollywogs). The following experiment gives this hypothesis an additional proof.

[2] In the experiments 1 and 3 the numbers showing a distinct result of induction are placed in parenthesis.

6. In passing through a vertical 30 $\mu$ wide slit, the ray beam does not show any trace of diffraction. As is seen in the table, two central sections are affected by the induction in this case. If we take into consideration the shrinkage due to the imbedding of the root tip, this corresponds approximately to a layer 25–28 $\mu$ in thickness. The shortest light rays (of approximately 0.4 $\mu$) under these circumstances would give a distinct diffraction, and the root (as has been pointed out under 5) certainly would be markedly influenced. Common ultraviolet rays hardly could play a rôle in this phenomenon, as they are not noticeably absorbed by water. I may add that preliminary experiments with the photographic plate have given a negative result.—ALEXANDER GURWITCH, *University of Simferopol, Russia.*

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## EFFECT OF LIGHTNING ON TRUNK OF PLATANUS OCCIDENTALIS

### (WITH ONE FIGURE)

In the spring of 1924, a large sycamore tree in a neighbor's yard was struck by lightning. The trunk of this tree was smooth and without branches for a distance of about 20 feet. The lightning stroke did no more damage than to remove some of the outer bark, leaving well marked paths down the trunk. There was no visible tearing or splitting of the trunk or branches. With the beginning of the growing period, however, the trunk began to develop numerous buds all the way from the branches of the large crown down to the ground. The twigs developed on all sides, although not uniformly, both on the lightning paths and on the undisturbed part of the bark. Over 200 twigs developed on the trunk, as shown in fig. 1.

This event may indicate methods of inducing active growth or rejuvenation of plant tissues which have been differentiated into a condition of stability. This tree for years had produced no buds on the trunk, but as a result of the lightning stroke it was thrown into an extraordinary activity of bud development from a previously stabilized tissue. The immediate cause at present can only be surmised. The lightning may have produced minute breaks or tearing of the cells of the cambium, or the electric charge may have produced direct changes in the protoplasm of the cells, or perhaps the development of buds was due to both causes.

Rejuvenescence and the renewing of the activity of reproduction and growth are bound to become problems of primary importance in the

physiology of the future, and it would be wise to begin the observation of
all probable causes of rejuvenation and dedifferentiation, in order to begin
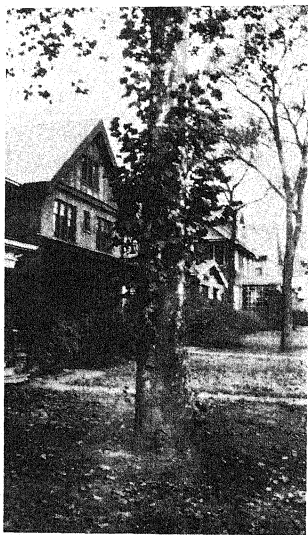


FIG. 1.—*Platanus occidentalis* with over 200 twigs developed on trunk in one
season, as result of stroke of lightning; photograph by C. K. KAO.

an attack on this interesting and important problem.—JOHN H. SCHAFF-
NER, *Ohio State University, Columbus, Ohio.*

# CURRENT LITERATURE

## NOTES FOR STUDENTS

**First sugar of photosynthesis.**—During the last thirty years, several English chemists and plant physiologists have put forward the theory that cane sugar is the first synthetic sugar resulting from the action of solar energy upon carbon dioxide and water. The only supporting evidence for such a hypothesis is the fact that there is a decided diurnal fluctuation in the amount of disaccharide sugar in the leaves of plants, low at night and increasingly high by day to a maximum in the afternoon, whereas the monosaccharide hexoses are fairly constant throughout the same period.

It is only fair to American plant physiologists to say that this cane sugar hypothesis has never been taken seriously on this side of the Atlantic, preference having been given always to the hypothesis that dextrose is the first sugar. It is a satisfaction, nevertheless, to have PRIESTLEY[1] reinterpret the older work of BROWN and MORRIS, and the more recent work of PARKIN, DAVIS, DAISH, and SAWYER, and reach the conclusion that the data really support the formation of hexoses as the primary photosynthetic sugars.

The difficulties involved in an explanation of the rôle of cane sugar in plants is well set forth in PRIESTLEY's discussion. He proposes a rather difficult solution of a difficult problem, namely, that cane sugar is not a storage product arising from enol formation and ultimate union of dextrose and levulose, nor does it arise directly from starch hydrolysis, enol formation, and synthesis. He prefers to believe that cane sugar is a product of intimate cellular metabolism by differentiating tissues. Thus the carbohydrate reserves, like starch or inulin, or the direct primary photosynthetic sugars, would be consumed in the active synthetic metabolism of the meristematic regions, and cane sugar would arise in some intricate way as a byproduct of this utilization of the reserves. Subsequently the cane sugar might be lost from the cells as the latter reach their elongation and maturation phases through vacuolation and differentiation, as possibly happens in cases where cane sugar appears in the sap flowing from decapitated vines and trees.

PRIESTLEY puts his views forward merely as a working hypothesis. Certainly the subject needs much careful investigation, and we need more adequate methods of recognition of the various sugars in mixtures, and particularly of distinguishing disaccharides from one another, and from glucosides. One would ultimately like to have an explanation which fits adequately to the main sources of cane sugar, the sugar beet and sugar cane.—C. A. SHULL.

[1] PRIESTLEY, J. H., The first sugar photosynthesis and the rôle of cane sugar in the plant. New Phytol. 23: 255-265. 1924.

**Hydrogen-ion concentration and vegetation.**—An effort to relate vegetation more exactly to soil acidity is seen in several recent studies that follow somewhat similar lines to those developed by KURZ.[2]

STRØM[3] examined numerous bodies of water in Norwegian mountains, and found their $P_H$ values to range from 3.8 to 8.5. Typically aerated localities gave an average $P_H$ value of 7.6, and several stagnant habitats gave an average of 4.5, showing that aeration is here the most important factor in controlling acidity. Under subarctic conditions, the majority of even stagnant localities are so well aerated that they show a neutral or only slightly acid reaction. The specific reaction of the water is regarded as a potential factor in determining the character of the vegetation, and more especially that of the algal flora.

Dealing more particularly with upland vegetation, CHODAT[4] has reported a detailed study of the soils of many of the plant associations of eastern France and Switzerland. He lays emphasis on his conclusion that each plant seems to grow in soils having a certain obtainable range of $P_H$ values, and that this amplitude of accommodation to the soil reaction, expressed in $P_H$ values, should replace such terms as calcifuge, calcicole, etc. Plant associations on soils of similar $P_H$ values he regards as homologous, and several such groups are distinguished.

BRAUN-BLANQUET[5] has determined the H-ion concentration of the soils of many of the garique associations of southern France, and finds that it varies little from the neutral ($P_H$ 7). While he regards the acidity of the soil, its calcium carbonate content, and its colloidal properties as important factors influencing the distribution of plants, the first seems inadequate to serve as a means of classifying the plant associations designated as garique.

In a more general discussion, SALISBURY[6] emphasizes the facts that the causes of soil acidity are various, that the reaction of an undisturbed soil is fairly constant throughout the year, and that the soil reaction is only one of many factors which determine the nature of the plant covering. He plots frequency curves with the $P_H$ values as abscissae and the number of localities as ordinates for several plants. These curves for the different species do not ex-

[2] KURZ, H., Hydrogen-ion in relation to ecological factors. BOT. GAZ. **76**:1–29. 1923.

[3] STRØM, K. M., $P_H$ values in Norwegian mountains, and their bearings upon the classification of freshwater localities. A reconnaissance. Nyt. Mag. Naturvidensk. **62**:237–244. 1925.

[4] CHODAT, F., La concentration en ions hydrogène du sol et son importance pour la constitution des formations végétales. Université de Genève, Thèse no. 748. pp. 115. 1924.

[5] BRAUN-BLANQUET, J., Études sur la végétation méditerranéene. III. Concentration en ions H et calcimétrie du sol de quelques associations de la garigue languedocienne. Bull. Soc. Bot. France **71**:639–647; 879–891. 1924.

[6] SALISBURY, E. J., The incidence of species in relation to soil reaction. Jour. Ecol. **13**:149–160. 1925.

hibit modes corresponding to the same $P_H$ value as they would if they were determined by the frequency of soils of a particular reaction, and some of the species exhibit a bimodal form, whereas the soil curve is monomodal. He also discusses the importance of a ratio of potassium to calcium as a factor in distribution, and concludes that the reaction of the soil as expressed in $P_H$ values is much more significant, being one of several important factors that govern the distribution of plant species under natural conditions.—GEO. D. FULLER.

**Specificity of enzymes.**—The specificity of enzyme action among the carbohydrate splitting enzymes has been much studied, and such action clearly demonstrated, but it has not been so clear that the proteolytic enzymes were similarly specific for the proteins which they usually hydrolyze. BLAGOVESCHENSKI[7] has investigated the action of proteases on plant globulins, using globulins from *Cannabis sativa, Brassica Rapa, Lupinus luteus, Phaseolus Mungo, Dolichos melanophthalmus, Hibiscus esculentus,* and *Lathyrus sinensis.* Using the protease derived from some one plant, its rate of reaction on the globulin from the same species, and on globulins from the other species, was compared by following the changes in amino-nitrogen during the reaction. Every protease was found to split the globulin of its own species more actively than those from any of the other species.

A study of the specific conditions of enzyme action of leaves has been made by BLAGOVESCHENSKI[8] and his coworkers, SOSSIEDOV[8], and BIELOZERSKI.[9] For the leaf invertases it has been found that each species of plant has a different optimal hydrogen-ion concentration, and that variation in percentage of hydrolysis with change in $P_H$ is of a very specific nature. Thus in the leaves of *Vitis vinifera,* the optimum $P_H$ for its invertase is 5.0, in *Gossypium herbaceum* 5.6, in *Glycyrrhiza glabra* 4.5, and in *Pyrus communis* 6.2. Fourteen species are recorded, each showing specific differences in the hydrogen-ion concentration relations of its own invertase. The peptases of different plants likewise have specific optimal hydrogen-ion concentrations for their action upon peptone. The optimum values for several species are given. For *Pyrus Malus* 4.5, *P. communis* 5.8, *Juglans fallax* 6.7, *Syringa vulgaris* 7.7, *Ungernia Severzovii* 8.0.

These investigations are of interest from the standpoint of comparative physiology, and emphasize the specific nature of protoplasmic products. Just how far one may go in applying these results to the problem of origin of species remains to be seen. BLAGOVESCHENSKI suggests that the origin of systematic species seems to be reduced to a purely chemical problem of the evolution of the proteins and other chemical bodies in the protoplasm, such chemical evolution expressing itself in external form, as well as in internal physiological character

[7] BLAGOVESCHENSKI, A. V., On the specific action of plant proteases. Biochem. Jour. **18**:795–799. 1924.

[8] BLAGOVESCHENSKI A. V., and SOSSIEDOV, N. I., The specific conditions of action of leaf invertases. Biochem. Jour. **19**:350–354. 1925.

[9] BLAGOVESCHENSKI, A. V., and BIELOZERSKI, A. N., The specific conditions of action of leaf peptases. Biochem. Jour. **19**:355–356. 1925.

and behavior. This is probably putting the case too strongly, but it does seem certain that physiological behavior and differentiation is sensibly specific, and that no one can draw very wide conclusions from the study of one, or even several species of plants.—C. A. SHULL.

**Taxonomic notes.**—YAMADA[10] has reported on collections of marine Chlorophyceae made on the Island of Formosa. The 33 species listed belong to 18 genera, distributed among 7 families. Four new species are described in the following genera: *Dictyosphaeria*, *Rhipidiphyllum*, *Cladophora*, and *Chlorodesmis*.

JOHNSTON[11] has contributed an investigation of the North American species of *Cryptantha* as the fourth paper in the studies of Boraginaceae. It is a very full record of all available material and bibliography. The general discussion includes the history, gross morphology, and generic relations of the genus. The author recognizes 57 species, grouped in 15 series, only 2 species being described as new. It is noteworthy that 35 of the species are credited to GREENE.

NAKAI[12] has published the results of a critical study of certain Japanese ferns. He considers 31 species in 8 genera, 5 of the genera represented each by a single species. The large genera are *Woodwardia*, with 11 species, one of which is new; *Polystichum*, with 10 species and 10 varieties; and *Pteridium*, with 5 species and 5 varieties.

MACBRIDE[13] has published an account of South American plants, mostly from the Captain Marshall Field expedition to Peru in 1922 and 1923. He includes 14 genera, represented by 29 species, 8 of which are new. Among them *Psoralea* is represented by 6 species, 4 of which are new. The same publication includes 2 new species of *Canavalia* by PIPER, and 2 new species of *Tithymalus* (Euphorbiaceae) by MILLSPAUGH.

PIPER[14] has undertaken to clarify the confusion that has existed in reference to the genus *Canavalia* (Leguminosae). As a result of the study of American species, the genus *Wenderothia* is restored, including 12 species, one of which is new; and *Canavalia* includes 26 species, 13 of which are new.—J. M. C.

**Haustoria of dodder.**—In a very detailed study, ZENDER[15] has examined the manner in which *Cuscuta europaea* penetrates its hosts, using material from

[10] YAMADA, YUKIO, Studien über die Meeresalgen von der Insel Formosa. Bot. Mag. Tokyo **39**:77–95. *figs. 5.* 1925.

[11] JOHNSTON, I. M., The North American species of *Cryptantha*. Contrib. Gray Herb. N.S. no. 74. pp. 114. 1925.

[12] NAKAI, T., Critical notes of Japanese ferns with special references to the allied species. Bot. Mag. Tokyo **39**:101–121. 1925.

[13] MACBRIDE, J. F., South American plants. Field Mus. Nat. Hist. Publ. 231. Bot. Series **4**:79–95. 1925.

[14] PIPER, C. V., The American species of *Canavalia* and *Wenderothia*. Contrib. U.S. Nat. Herb. **20**:555–588. 1925.

[15] ZENDER, J., Les Haustoriums de la *Cuscuta* et les réactions de l'hôte. Thèse no. 757, Université de Genève. pp. 81. 1924.

families extending from the Pteridophytes to the Compositae. Many variations in the haustoria are described and figured, but the only reaction on the part of the host is the development of some little wound tissue in a few plants such as *Rubus idaeus* and *Chaerophyllum silvestre*. Active chemical metabolism is indicated by the enlarged nuclei of the haustoria. The disorganization of the host tissues is shown[16] to be similar to that caused by parasitic fungi. Special attention is given to the behavior of haustoria in the xylem and phloem regions. In the former the conductive elements of the parasite apply themselves very closely to those of the host, fitting accurately into the pores and about the thickenings of the tracheae. They are apparently readily able to absorb water and dissolved mineral salts very soon after their entrance. Approaching the phloem the haustoria seem stimulated to greater extension, and send out digitately branched processes which enter the sieve tubes and companion cells, indicating that their contents are used in the nutrition of the parasite.

This study seems to be carefully made, well illustrated by drawings, and of a nature that makes a substantial advance in our knowledge of the subject.— GEO. D. FULLER.

**Botany and weeds.**—A recent publication[17] demonstrates well the advantages of attacking an economic problem by scientific methods. *Cyperus rotundus* is one of the most troublesome weeds of moist tropical soils. In India, where it is known as the "lavala weed," it reduces the annual yield of fertile tracts 25–30 per cent. Its multiplication by seeds, tubers, and rhizomes have made it particularly difficult of eradication.

A committee appointed by the Director of Agriculture of India, and charged with devising methods of control, decided to proceed by making a thorough botanical study of the plant rather than by the usual method of large scale experiments in the field. The results as expressed in this bulletin include a careful taxonomic, anatomical, and ecological study of all phases of its life history, with particular attention to its various methods of propagation. This includes many experiments on seed germination and planting of tubers, all yielding interesting results, that are reported in detail and are important in leading by various routes to the vulnerable point in the life history of the weed, that is, to the conditions necessary for the development of the seeds and tubers.

From these data it has been possible to formulate at once a program of eradication that will doubtless be more efficient than any indicated by field experiment alone. This consists in a combination of fallowing during the dry season and cultivation during the rainy months. The results have thus entirely justified the method of first studying the plant, and only later dealing with field practice.—GEO. D. FULLER.

[16] ZENDER, J., Les Comportements des haustoriums du *Cuscuta europaea* dans les tissus de la plante parasite. Compt. Rend. Soc. Phys. et Hist. Nat. Genève 41:131–134. 1924.

[17] RANADE, S. B., and BURNS, W., The eradication of *Cyperus rotundus* L., a study in pure and applied botany. Mem. Dept. Agric. India, Bot. Series 13:99–192. 1925.

# THE

# BOTANICAL GAZETTE

## PACIFIC COAST SPECIES OF LATHYRUS

### R. V. BRADSHAW

### (WITH TWENTY-NINE FIGURES)

Since the publication of WHITE's revision of the North American
species of *Lathyrus* in 1894, the species of the Pacific states have had
no general treatment. A monograph of the entire genus, showing the
relationships of the various species, is certainly needed; but the
greatest need for such a work, on account of the confusion among
the various species, seems to have existed for the Pacific states. Of
the botanists since the time of WHITE, perhaps C. V. PIPER[1] has
done the most toward restoring order, in so far as some species are
concerned. That paper, however, did not include all the species,
and several have been described since the revision, and extensive
collections of material have been made; hence it seems desirable to
prepare a more general revision of the species of the three coast
states. This is only an attempt, however, since even at this date the
region is in places botanically unknown, and an exposition of certain
forms must as yet be undertaken with much hesitation. PIPER
elucidated those subspecies related to *L. pauciflorus*. A similar
treatment is needed for those allied to *L. splendens* and *L. Bolanderi*.

In some species, such as *L. Bolanderi*, the variation is so great
that scores of forms might be described, but I cannot see the value
of it at present. Further investigation will be necessary to determine
their importance, which being done should be included in a detailed

[1] Proc. Biol. Soc. Wash. 31: 189–197. 1918.

monograph. Accordingly no attempt has been made to name these numerous and perhaps inconstant variations, as, for example, the prostrate form with narrow leaflets and small stipules; the dwarfed erect form with dilated stipules and densely puberulent herbage; the form with broad, thin, glabrous leaflets and minute stipules; and the form with coriaceous leaflets and small stipules.

There is often a very noticeable variation in the leaflets, stipules, tendrils, color of the flowers, and appearance of the plant. This is due to ecological and other environmental conditions. There seems to be a tendency for plants with narrow leaflets to have small and narrow stipules, while in the same species those with broad leaflets have usually larger and more dilated stipules. Pubescence, while varying in many cases, is most often constant. The shape as well as the length of the calyx teeth is one of the most important and practically constant characters, and is of great diagnostic value in this genus, although it has never been given sufficient attention.

WHITE, in his key, overemphasized the importance of the length of the tendrils, and also the distinction between climbing and erect plants. It is true that species like *L. Cusickii* and *L. rigidus* are always erect; yet it is equally true that species like *L. Lanszwertii* and *L. pauciflorus* may be either erect or climbing. The climbing forms have well developed tendrils, while the erect ones have minute or no tendrils. Of the literature of the genus, preceding the work of WHITE,[2] can be mentioned that of ALEFELD[3] and that of WATSON.[4] In the last paper, which included all the then known species of North America, but thirteen were listed; WHITE's revision contained thirty-three.

Besides my own herbarium, I have had the use of specimens of the National Herbarium, University of California, Stanford University, University of Oregon, and the California Academy of Sciences. I am indebted to the curators of these herbaria, and also to J. C. NELSON, M. E. PECK, Miss MARY A. DAY, and the Director of the Royal Botanic Gardens at Kew for data, and to Dr. ABRAMS for assistance and advice. In the lists of specimens examined, the following abbreviations are used: RVB, my own herbarium; UO, Uni-

[2] Bull. Torr. Bot. Club 21:444–458. 1894.

[3] Bonplandia 9:146. 1861.                    [4] Proc. Amer. Acad. 11:133–135. 1876.

versity of Oregon; UC, University of California; CA, California
Academy of Sciences; DS, Dudley Herbarium of Stanford Uni-
versity; and US, United States National Herbarium.

LATHYRUS (Tourn.) L., Sp. Pl. 729. 1753.

Herbs climbing with well developed tendrils which terminate the
leaves, or the tendrils much reduced and the plants erect; stipules
foliaceous, as large as the leaflets or small and inconspicuous; leaves
pinnate, with 2–20 leaflets, but with *L. aphaca* the leaflets are want-
ing, and the enlarged stipules assume the appearance of simple
opposite leaves, the tendril projecting between them; flowers one
to many in axillary racemes; calyx teeth 5, the 3 lower teeth usually
longer than the 2 upper; corolla papilionaceous, campanulate at
base; stamens diadelphous (9 and 1), monadelphous at base; ovary
sessile or stalked; ovules usually numerous; style transversely flat-
tened, curved and bent at right angles with ovary, dilated at summit
and bearded on inner face for $\frac{1}{3}$–$\frac{1}{2}$ its length; pods flat or terete,
2-valved and 1-celled.

About 200 species, natives of South America and the northern hemispheres.
Type species, *Lathyrus sativus* L.

KEY TO SPECIES AND SUBSPECIES

Leaflets 0–2; introduced species
    Leaflets 0; stipules large, resembling leaves; flowers yellow. 1. *L. aphaca*
    Leaflets 2; stipules and flowers otherwise
        Plants annual; flowers small, pink or red
            Pods hirsute; plants tall......................2. *L. hirsutus*
            Pods glabrous; plants small and slender...........3. *L. sphaericus*
        Plants perennial; flowers large, white or pink.........4. *L. latifolius*
Leaflets 2–20; native species
    Plants densely silky villous........................5. *L. littoralis*
    Plants not densely silky villous
        Peduncle not longer than rachis of its leaf, or if so either the plants erect,
            less than a meter in height, or some of calyx teeth rather wide and
            longer than tube; or the flowers yellow; plants erect or climbing not
            over a meter high unless stems are winged or calyx teeth longer than
            calyx tube and about 2 mm. broad; flowers 10–25 mm. long
        Calyx teeth usually shorter than calyx tube, or if longer either the
            plants with peduncles bearing one flower, or the teeth which exceed
            the tube very slender, not 2 mm. broad; flowers purple, blue, white,

or yellow; if yellow then the racemes with 2–7 flowers, or if as many
as 11 flowers the stipules not one-half the size of leaflets
Flowers white or yellow
  Leaflets narrowly lanceolate, much longer than wide
                                         6. *L. Cusickii*
  Leaflets oblong, lanceolate or obovate, 2–4 times as long as wide
    Leaflets oblong or obovate, thin to coriaceous; stipules minute
      to one-half as large; flowers yellowish
      Stipules minute
        Flowers 10 mm. long; leaflets coriaceous....11. *L. Tracyi*

        Flowers 20 mm. long; leaflets thin to coriaceous
                              10. *L. nevadensis*
      Stipules $\frac{1}{3}$–$\frac{1}{2}$ size of leaflets.....10a. *L. nevadensis stipulaceus*
    Leaflets oblong to linear, very veiny and coriaceous; stipules
      nearly as large; flowers white................7. *L. rigidus*
Flowers purple or blue
  Leaflets usually 4; stems not winged
    Leaflets oval or oblong.....................8. *L. bijugatus*
    Leaflets narrowly linear...........8a. *L. bijugatus Sandbergii*
  Leaflets 2–14; stems winged or without wings
    Stipules small, except in 10a and 12, not one-half so large as
      adjacent leaflets
      Stems not winged
        Flowers 15–30 mm. long; leaflets about twice as long as
          wide; plants not villous
          Flowers 15–16 mm. long, purplish to red; leaflets usually
            in 4 pairs
            Leaflets elliptical....................9. *L. Nuttallii*
            Leaflets lanceolate.......9a. *L. Nuttallii lanceolatus*
          Flowers 20 mm. long, purplish to yellow; leaflets usually
            in 3 pairs
            Stipules minute..................10. *L. nevadensis*
            Stipules $\frac{1}{3}$–$\frac{1}{2}$ size of leaflets
                10a. *L. nevadensis stipulaceus*
         Flowers 15 mm. long or less; leaflets usually 3 or 4 times
          as long as wide, or else the plant villous and the
          flowers 1 or 2 in a raceme
          Flowers usually 1...................12. *L. Torreyi*
          Flowers more than 2
            Plants tall; tendrils divided, sometimes well devel-
              oped; leaflets various........13. *L. Lanszwertii*
           Plants low, erect; tendrils minute; leaflets linear
               13a. *L. Lanszwertii aridus*

Stems winged; see nos. 17, 18, and 19
Stipules large, frequently one-half size of leaflets except in
   plants with narrow leaflets
Leaflets coriaceous, 6–12; flowers 3–5
   Flowers 18–20 mm. long[5]
      Leaflets broader than linear-lanceolate
         Leaflets elliptic to ovate-lanceolate, acute
                                14. *L. pauciflorus*
         Leaflets oval to ovate, obtuse
                       14a. *L. pauciflorus utahensis*
      Leaflets linear to linear-lanceolate
                      14b. *L. pauciflorus tenuior*
   Flowers 12–16 mm. long
      Leaflets oval, elliptic or ovate
                  14c. *L. pauciflorus Schaffneri*
      Leaflets linear to narrowly oblong
                14d. *L. pauciflorus Brownii*
   Leaflets flaccid, 10–20; flowers 6–10 . . . . . 15.  *L. polyphyllus*
Calyx teeth usually longer than calyx tube, or, if shorter, the flowers
  yellow and borne 7–19 in the raceme and with dilated stipules, one-
  half size of leaflets; flowers white, purple, or blue; calyx teeth when
  long, rather broad, about 2 mm. wide
Flowers purplish to white or tawny
  Stems not winged, or if so the leaflets not fleshy
    Stems not winged
      Stipules large, often one-half size of leaflets . . 16.  *L. Bolanderi*
      Stipules small or minute
        Leaflets with white appressed hairs; flowers tawny
                  16a. *L. Bolanderi quercetorum*
        Leaflets puberulent; flowers blue, white, or tawny
                  16b. *L. Bolanderi violaceus*
  Stems frequently winged
    Leaflets 8–12, pubescent or glabrous; flowers 6–15
      Flowers rose purple, not fading to yellow; leaflets glabrous,
        somewhat glaucous . . . . . . . . . . . . . . . . . .17.  *L. Jepsonii*
      Flowers white, veined with purple, fading to yellow;
        leaflets puberulent to velvety pubescent
                      18. *L. Watsonii*
    Leaflets 2–7, pubescent or glabrous; flowers 2–6
                     19.  *L. palustris*
  Stems never winged, leaflets fleshy . . . . . . . . . .20.  *L. maritimus*

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[5] This key to the subspecies of *L. pauciflorus* has been adapted from PIPER's paper.

Flowers yellowish
    Leaflets thin, membranaceous..............21. *L. ochropetalus*
    Leaflets thick, coriaceous....................22. *L. sulphureus*
Peduncle longer than rachis of its leaf; calyx teeth narrow, not so long as
    or scarcely exceeding the tube; plants several meters in length,
    climbing; flowers white, blue, or reddish, never yellow; flowers
    20–35 or more mm. long; stems never winged
  Flowers 3–3.5 cm. long, red or purple..............23. *L. splendens*
  Flowers 2–2.5 cm. long, blue, purple, or white
    Flowers white................................24. *L. laetiflorus*
    Flowers not white....................24a. *L. laetiflorus Alefeldi*

1. LATHYRUS APHACA L., Sp. Pl. 729. 1753.—Annual, entirely glabrous; stems slender and climbing, 20–40 cm. high; stipules large, entire, broadly heart-shaped, resembling simple opposite leaves; leaflets none; tendrils well developed, about 2–3 cm. long, scarcely longer than stipules; peduncles slender, bearing 1 or 2 yellow flowers; flowers 6–9 mm. long; pods flat, 25 mm. long, containing 4–7 seeds.

This is one of the recently introduced species from Europe; it is well established along railroads and in gardens at Portland, Salem, and Eugene, Oregon. May–July. Type locality, "Habitat in Italia, Gallia, Anglia inter segetes." According to PARKER (Rhodora **23**:246. 1921), *L. nissolia* L. has been collected at Pullman, Washington. If found in the field it can readily be recognized by its pale red and rather small flowers, and leaves which are reduced to long, slender, flattened leaf stalks, without stipules. I have not examined a specimen from our limits.

OREGON.—Lane County: Eugene, May 1914, Mrs. *Norman*, Misses *Sanborn* and *Howell* (UO); same, May 1919, *Bradshaw* 1083 (RVB); same, May 18, 1919, *Bradshaw* (RVB); Multnomah County: Eastside, Portland, May 1919, *Nelson* 2592 (DS).

2. LATHYRUS HIRSUTUS L., Sp. Pl. 732. 1753.—Annual, the younger shoots somewhat hairy; stems branching at base, slender, 30 cm. or more high; margins of stems with prominent wings; stipules small and very narrow, hardly over 15 mm. long, linear-lanceolate; leaflets 2, linear-lanceolate, about 7 cm. long by 1 cm. wide; tendrils well developed, branching; peduncles longer than leaves, rather slender, bearing 1 or 2 small red flowers; flowers 7 mm. long; calyx teeth unequal, lower linear-lanceolate, upper deltoid, nearly as long as tube; pods flat, very hairy, about 4 cm. long; seeds 6–8.

This species was introduced at Salem, Oregon, on neglected parkings in 1919, where it has become well established. May. Type locality, "Habitat inter Angliae, Galliae segetes."

OREGON.—Marion County: Salem, July 1919, *Nelson* 2727 (DS).

3. LATHYRUS SPHAERICUS Retz., Obs. **3**:39. 1783.—Annual, perfectly glabrous; stems very slender, branching, 15–25 cm. high; stipules small, linear to subulate, 7–8 mm. long; leaflets 2, linear, 2–4 cm. long; tendrils represented only by short bristle; peduncles slender, 5–6 cm. long, bearing 1 small dark red flower; flowers about 8 mm. long; calyx teeth nearly equal in length, lowest linear-subulate, others linear, all longer than tube; pods 3–4 cm. long, glabrous; seeds 6–11.

This introduced annual has been collected at Salem, Oregon, where it was found on parkings. May. The type locality was not given with the original description.

OREGON.—Marion County: Salem, May 1919, *Nelson* 2534 (DS).

4. LATHYRUS LATIFOLIUS L., Sp. Pl. 733. 1753.—Perennial, perfectly glabrous; stems stout, climbing, very tall and coarse, petioles and stem broadly winged; stipules large and veiny, semi-sagittate, acute, sparingly serrate; leaflets 2, coriaceous, oblong-lanceolate, about 8 cm. long; tendrils as long as leaves, divided; peduncles sometimes 25 cm. long, of much greater length than leaves; flowers 6–10, pink or white, over 20 mm. long; calyx teeth glabrous, lowest subulate, upper triangular and very short; pods 5–8 cm. long.

This species is often planted in gardens, and is frequently met with as an escape in waste ground. Once established it is well able to hold its own. June–July. Type locality, "Habitat in Europae sepibus."

OREGON.—Lane County: Eugene, August 1920, *Bradshaw* 1953 (RVB); Marion County: Salem, June 1915, *Nelson* 270 (DS).

CALIFORNIA.—County not determined: Feather River region, June 1920, *Anna Head* (CA).

5. LATHYRUS LITTORALIS (Nutt.) Endl., Walp. Rep. **1**:722. 1842.—*Astrophia littoralis* Nutt. T. and G., Fl. N. A. **1**:278. 1838; *Orobus littoralis* Gray, Pacif. R.R. Rep. **4**:58. 1856.—Perennial, the entire plant densely silky villous; stems numerous, stout, decumbent, 15–65 cm. long; stipules more than twice the size of leaflets, ovate to oblong, entire; leaflets 2–10, linear-spatulate, 5–20 mm. long; tendrils none, leaf ending in small linear leaflet; peduncles

stout, 4–9 cm. long, greatly exceeding leaves; flowers 4–10, purple
and white, about 20 mm. long; calyx teeth lanceolate, as long as
tube, densely villous; pods 30 mm. long by 10 mm. broad, villous;
seeds 3–5.—Fig. 10.

Along sea coasts, Humid Transition Zone, Washington, Oregon, and Cali-
fornia. April–August. Type locality, "Sand hills of the estuary of the Oregon."

WASHINGTON.—Chehalis County: Laidlaw, May 1897, *Lamb* 1119 (DS);
Island County: Whidby Island, *Gardner* (UC).

OREGON.—Clatsop County: Clatsop, August 3, 1887, *Henderson* 235 (DS);
Clatsop Beach, July 1916, *Nelson* 819 (DS); Tillamook County: Tillamook Bay,
July 1882, *Howell* (UO).

CALIFORNIA.—Humboldt County: Eureka, April 1918, *Paulson* (DS);
Humboldt Bay, May 1906, *Tracy* 2451 (DS); Samoa, 1913, *Hutchinson* (CA);
Marin County: Mt. Tamalpais, May 1891, *Cannon* (CA); Pt. Reyes, May 1906,
*Eastwood* (CA); San Francisco County: Lake Merced, June 1887, *Greene* (DS,
UC); near Cliff House, San Francisco, May 1902, *Baker* 695 (UC); Santa
Cruz County: Watsonville, June 1903, *Elmer* 4390 (DS, CA); San Mateo
County: Salida Beach, July 1918, *Brandegee* (UC); Halfmoon Bay, June 1905,
*Brandegee* (UC).

6. LATHYRUS CUSICKII S. Wats., Proc. Amer. Acad. **17**:371.
1882.—Perennial, glabrous or slightly pubescent; stems slender,
erect, branching, about 15–30 cm. high; stipules small, not half so
long as adjacent leaflets, semi-sagittate, acuminate; leaflets 4–6,
linear lanceolate to narrowly linear, 3–6 cm. long, acute or nearly
so; tendrils none, sometimes a minute bristle present; peduncles
slender, equaling or exceeding length of leaves, bearing 2 or 3 white
flowers; flowers 20 mm. long; calyx teeth glabrous, linear, almost
equal, not so long as tube; pods 4–5 cm. long by 6 mm. wide.—Fig. 17.

Dry mountain slopes, eastern Oregon; Canadian Zone. May–June. Type
locality, "On dry mountain slopes, Union County, Oregon."

OREGON.—Eastern Oregon: dry forests, May 1882, *Cusick* (UC); Umatilla
County: Blue Mountains, near Meacham, May 1908, *Cusick* 3236 (UO, DS);
Union County: June 1882, *Cusick* 193 (UO, US); Imnaha National Forest:
Chico Station, May 1907, *Coville* 2337 (US).

7. LATHYRUS RIGIDUS White, Bull. Torr. Bot. Club **21**:455.
1894.—*L. albus* S. Wats., Bot. Calif. **2**:442. 1880.—Perennial,
glabrous and somewhat glaucous; stems stout, numerous from
thick rootstalk, 20–30 cm. high; stipules nearly as large as leaflets,
semi-sagittate, lanceolate, acuminate; leaflets 6–10, linear-oblong,

veiny and rigid like stipules, 1–2 cm. long, acute at both ends; tendrils none, a minute bristle terminating rachis of leaf; peduncles stout, equaling or exceeding leaves, bearing 2 or 3 flowers; flowers white, 15–20 mm. long; calyx teeth linear to deltoid, unequal, shorter than tube; pods glabrous, 3–3.5 cm. long by 7 mm. wide; seeds about 3.—Fig. 24.

Eastern Oregon, in the Blue Mountains to northern California; Arid Transition Zone. May–July. Type locality, Union County, Oregon.

OREGON.—Eastern Oregon, May 1897, *Cusick* 1604 (DS); Blue Mountains, May 1895, *Howell* (DS); Crook County: Willow Creek, June 1894, *Leiberg* 293 (DS, UO); Malheur County: May 1896, *Leiberg* 2117 (DS, UO, CA); Union County: April–May, 1910, *Cusick* 3428 (UO).

CALIFORNIA.—Modoc County: Goose Lake Valley, May 1894, Mrs. *Austin* (DS); Acabe Plains, 1894, Mrs. *Austin* 45 (UC); near Fort Bidwell, May 1904, Mrs. *Manning* 19 (UC); Davis Creek, June 1893, *Laura Black* 80 (DS).

8. LATHYRUS BIJUGATUS White, Bull. Torr. Bot. Club **21**:457. 1894.—Perfectly glabrous throughout; stems erect, rather slender, branching from base, 10–30 cm. high; stipules small, not half so large as adjacent leaflets, linear, subulate, semi-sagittate; leaflets about 4, oval to oblong or obovate, acute or obtuse, somewhat coriaceous, rather thin, paler beneath, 2–4 cm. long; tendrils very much reduced, represented by minute bristle; peduncles slender, half as long as leaves, bearing 2 or 3 purplish flowers; flowers 10 mm. long; calyx teeth nearly equal, triangular, about one-fourth as long as calyx tube; pods compressed; seeds about 6.—Fig. 15.

Eastern Washington and Idaho; Arid Transition Zone. May–June. Type locality, Latah County, Idaho.

WASHINGTON.—Spokane County: Hangman Creek, May 1893, *Sandberg and Leiberg* 24 (US, UC, CA); Whitman County: Pullman, May 1897, *Elmer* 214 (US).

8a. LATHYRUS BIJUGATUS SANDBERGII White, Bull. Torr. Bot. Club **21**:457. 1894.—*L. Sandbergii* Howell, Fl. N.W.A. **1**:160. 1898.—This differs from the species in having narrowly linear or linear-lanceolate leaflets, 3–11 cm. long.

Eastern Washington and Idaho; Arid Transition Zone. May–June. Type locality, Latah County, Idaho.

WASHINGTON.—Whitman County: Pullman, May 1909, *Duthie* 15 (DS); same locality, June 1893, *Piper* (DS).

9. LATHYRUS NUTTALLII S. Wats., Proc. Amer. Acad. 21:450. 1886.—*L. venosus* Muhl. var. ♂ T. and G., Fl. N.A. 1:274. 1838.—
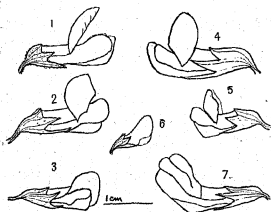


FIGS. 1–7.—Fig. 1, *L. Watsonii;* fig. 2, *L. palustris;* fig. 3, *L. Nuttallii;* fig. 4, *L. Jepsonii;* fig. 5, *L. Lanszwertii;* fig. 6, *L. Lanszwertii aridus;* fig. 7, *L. nevadensis.*

Perennial, herbage sparingly pubescent throughout; stems rather slender, 40–90 cm. high, branching; stipules narrow, semi-sagittate, not half so long as adjacent leaflets, usually entire and long acuminate; leaflets 6–14, commonly 8, narrowly or broadly elliptical, acute, 2–5 cm. long; tendrils simple or divided, well developed but slender; peduncles slender, shorter or longer than leaves, bearing 4–6 reddish flowers; flowers 12–16 mm. long; calyx teeth unequal, not half so long as tube; pods oblong, glabrous, 25–40 mm. long; seeds several. —Fig. 3.

British Columbia to California in the coast region; Humid Transition Zone. May–July. Type locality, "Upper California."

This is somewhat similar to *L. nevadensis*, which is perhaps its nearest relative, but differs in the number of leaflets, and in the size and color of the flowers; *L. Nuttallii* has leaflets usually in 4 pairs, while *L. nevadensis* has leaflets generally in 3 pairs. The flowers of *L. Nuttallii* are reddish purple, while those of *I*. *nevadensis* are blue and white varying to yellow. *L. Nuttallii* is a taller plant and grows at a lower and more constant altitude.

WASHINGTON.—Chelan County: Chiwaukum, June 1904, *Whited* 2528 (UO); Clarke County: La Camas, June 1884, *Henderson* (DS); Kittitas County: Cle Elum, May 1897, *Whited* 364 (US); Klickitat County: Falcon Valley, April–August 1885, *Suksdorf* (UC); Pierce County: Tacoma, June 1920, *Eastwood* 9642 (CA); Skagit County: Weaverlings near Anacortes, May 1919, *Roush* (DS).

OREGON.—Sauvie's Island, May 1886, *Howell* 977 (UC); locality not determined: 1871, *Hall* (US); Lane County: Eugene, April 1920, *Bradshaw* 1364 (RVB); Multnomah County: Mt. Scott, May 1903, *Sheldon* 12012 (DS, UO).

CALIFORNIA.—Butte County: Stirling, May 1919, *Heller* 13162 (CA); Lake County: La Keport, May 1917, *Bentley* (DS); Siskiyou County: locality not given, July 1915, *Heller* 12091 (CA).

9a. LATHYRUS NUTTALLII LANCEOLATUS (Howell) Piper, Proc. Biol. Soc. Wash. 31:191. 1918.—*L. lanceolatus* Howell, Fl. N. W. A.

1:158. 1898.—This subspecies has oblong-lanceolate leaflets of membranaceous texture and lanceolate stipules; the stipules are larger than with the species.

Oregon in the coast region; Humid Transition Zone. Type locality, Glendale, Oregon.

OREGON.—Sauvie's Island, May 1886, *Howell* (DS).

*L. Peckii* Piper (Proc. Biol. Soc. Wash. **31**:190. 1918) was based on only one specimen collected by M. E. PECK of Willamette University. PECK writes that only one specimen was found, and that on succeeding visits to the type locality (Harbor, Curry County, Oregon) no other plants could be obtained; accordingly the original was probably a hybrid or unusual growth of the variable *L. Nuttallii.*

10. LATHYRUS NEVADENSIS S. Wats., Proc. Amer. Acad. **11**:133. 1876.—*L. venosus obovatus* Torr., Pac. R. R. Rep. **4**:77. 1857, in part; *Vicia nana* Kell., Proc. Calif. Acad. **7**:89. 1876; *L. obovatus* White, Bull. Torr. Bot. Club **21**:455. 1894.—Perennial, glabrous to sparingly pubescent; stems erect, slender or stout, frequently branched, 10–45 cm. high; stipules small, not half so large as leaflets, semi-sagittate, linear-lanceolate, acute to acuminate; leaflets 4–8, ovate to ovate-oblong or obovate, acute or obtuse, usually rather thin but sometimes coriaceous, 0.5–3 cm. long, glabrous or slightly pubescent; tendrils represented usually by minute bristle, but larger specimens often have long and more developed simple tendrils; peduncles shorter or longer than leaves, bearing 2 or 3 bluish or ochroleucous flowers; flowers commonly 20 mm. or more long; calyx teeth linear to linear-lanceolate, not so long as tube; pods glabrous, 3 cm. long.—Fig. 7.

*L. nevadensis* is very variable; certain forms are small with coriaceous leaflets, while others more closely approach *L. Nuttallii.* There are forms similar to *L. Tracyi,* which has numerous small flowers 10 mm. long; but these forms are unlike *L. Tracyi* in the characters of stipules, leaflets, and general appearance. Between *L. nevadensis* and the subspecies *stipulaceus* are to be found specimens with stipules of varying sizes. One sheet examined, collected by *Rattan* in 1886 in El Dorado County, California, has both the large and the small stipules on plants collected evidently at the very same station. The specimen with the large stipules has more numerous flowers. Two other specimens collected by *Butler* (no. 1288) and *Heller* (no. 12007) may be at least of subspecific value. Both specimens have 7–10 yellowish flowers 10 mm. long. The stipules are well developed but not large, and the herbage is somewhat glaucous. Further study in the field is needed to determine whether they are distinct species or subspecies of *L. nevadensis.*

WASHINGTON.—Locality not given, 1889, *Vasey* 252 (US); Cascade Mountains near upper valley of Nesqually, July 1897, *Allen* 297 (DS).

OREGON.—Localities not determined: July 1886, *Cusick* 185 (UC); mountains in shade, *Cusick* (UO); Eastern Oregon: dry open forests, May 1898, *Cusick* 1881 (US); Wallowa Mountains, May, *Cusick* 2392 (DS); Southern Oregon: Grave Creek Mountains, May 1887 *Henderson* 1343 (DS, UO); southern slope of Siskiyous, June 1899, *Leiberg* 4039 (UO); Benton County: Corvallis, top of Mary's Peak, June 1916, *Gilbert* 109 (US); Coos County: south fork of Coos River, March 1911, *Smith* 3591 (US); Josephine County: Jones Creek, Grants Pass, April 1913, *Dale* (DS); Klamath County: Lake of the Woods, July 1897, *Coville* and *Applegate* 84 (US); Linn County: Smith River, July 1903, *Sheldon* 12738 (UO); Umatilla County: Blue Mountains, near Meacham, May 1908, *Cusick* (DS); same locality and date, *Cusick* 3336 (UO); Wasco County: Oregon National Forest, west of Friend, June 1917, *Lawrence* 282 (DS).

CALIFORNIA.—Amador County: Panther Creek, May 1895, *Hansen* 1084 (DS); Butte County: Forest Ranch, 1898, Mrs. *C. C. Bruce* 2082 (DS); Calaveras County: shade of big trees, May 1895, *Davy* 1547 (UC); Mokelumne Hill, F. E. Blaisdell (CA); Del Norte County: French Hill, April–May 1906, *Eastwood* 74 (CA); El Dorado County, locality not given, 1886, *Rattan* (DS); Glenn County: west of Bennet Spring, June 1915, *Heller* 12007 (CA); Humboldt County: Buck Mountain, June 1913, *Tracy* 4172 (UC); Klamath River, June 1901, *Chandler* 1443 (UC); Mariposa County: Sherlocks, May 1903, *Congdon* (UC); Sweetwater Peaks, May 1911, *Hall* 8842 (DS); Napa County: Howell Mountain, May 1902, *Tracy* 1527 (UC); Placer County: Dutch Flat, April 1921, *Patterson* (DS); Forest Hills, April 1865, *Bolander* 4629 (UC); Siskiyou County: Goosenest foothills, June 1910, *Butler* 1588 (DS); Humbug Mountain, May 1910, *Butler* 1288 (DS, UC); near Marble Mountain, June 1901, *Chandler* (UC); Tehama County: Mineral, July 1920, *Clemens* (CA); Trinity County: Trinity Summit, May–August 1899, *Davy* and *Blasdale* 5833 (UC); Yosemite: June 1911, *Hall* 8986; Yuba County: Camptonville, April 1918, *Eastwood* (CA).

10a. **Lathyrus nevadensis stipulaceus** (White), n. comb.— *L. obovatus stipulaceus* White, Bull. Torr. Bot. Club **21**:455. 1894.— This differs from the species in having enlarged stipules, one-third or one-half size of leaflets, and in having up to 10 flowers in the raceme.

With the species from British Columbia to California in the Transition Zone. The type locality for the species is "Mammoth Grove and Duffield's Ranch," Calaveras County, California. The subspecies was collected between Colville and Spokane, Washington.

CALIFORNIA.—El Dorado County: 1886, *Rattan* (DS), specimen marked "a."

11. **Lathyrus Tracyi**, n. sp.—Sparingly pubescent, bright green, not glaucous; stems erect, 58 cm. high, angled but not winged; stipules narrowly semi-sagittate, linear-lanceolate, subfalcate, not half so large as adjacent leaflets, entire or rarely dentate, acuminate at apex; leaflets coriaceous, veins of under surface puberulent, 3 or 4 pairs, 2–5 cm. long, 6–10 mm. wide, tapering from middle, acute at both ends; petioles 1 mm. long; tendrils of lower leaves minute, those of upper leaves divided, 15 mm. long; flowers 10 mm. long, yellow or cream colored, 10 or 11 in raceme; calyx teeth unequal, upper 1 mm. long, lateral linear-lanceolate, 2 mm. long, lowest 2.5 mm. long, none longer than calyx tube; fruit not seen.

Grouse Mountain, near Janes' Ranch, Humboldt County, California, May 26, 1918, *Tracy* 4943 (UC 202014, the specimen marked "a").

This species is related to *L. nevadensis* and *L. Lanszwertii*, having foliage and stipules like the latter, and flowers and general appearance suggesting the former.

12. LATHYRUS TORREYI Gray, Proc. Amer. Acad. **7**:337. 1867.—
*L. Torreyi tenellus* Wiegand, Bull. Torr. Bot. Club **26**:135. 1899.—
Perennial, minutely villous; stems slender, erect, branching, 15–45 cm. high; stipules small, 8–10 mm. long, usually not half so large as adjacent leaflets, lanceolate, acuminate, semi-sagittate; leaflets 8–14, rather thin, paler beneath, ovate to oblong or lanceolate, 5–10 mm. long, acute or obtuse; peduncles very slender, 8–40 mm. long, much shorter than leaves; flowers usually 1, sometimes 2, bluish white and about 10 or 15 mm. long; calyx teeth unequal, upper triangular, lower 3 subulate and longer than tube; pods scarcely over 25 mm. long, linear-oblong, somewhat pubescent; seeds 3–5.—Fig. 12.

In open woods, Washington, Oregon, to central California, in the coast region; Humid Transition Zone. April–July. Type locality, Mendocino or south part of Humboldt County, California.

WASHINGTON.—Pierce County: Tacoma, June 1894, *Thompson* (DS); same locality, June 1894, *Flett* (UO).

OREGON.—Southern Oregon: Siskiyou Mountains, July 1899, *Dudley* (DS); Clackamas County: Eagle Creek, June 1884, *Henderson* (DS); Lane County: Fall Creek, May 1920, *Wynd* (RVB); Marion County: Silver Falls, June 1916, *Nelson* 663 (DS).

CALIFORNIA.—Humboldt County: Willow Creek, June 1918, *Abrams* 7098 (DS); Dows Prairie, May 1917, *Tracy* 4786 (UC); Kneeland Prairie, August 1909,

*Tracy* 3062 (UC); Eureka, April 1913, *Hutchinson* (CA); Marin County: near
Ross Valley, May 1904, *Baker* (UC); Bolinas Heights, June 1891, *Brandegee*
(UC); Lagunitas Lake, May 1918, *Eastwood* (CA); Mendocino County: Lynal
Ridge, June 1901, *Carruth* (CA); near Comtche, June 1906, *Walker* 271 (UC);
near Orrs Hot Springs, May 1921, *Head* (CA); Walkirs Valley, Willets, May
1899, *Davy* and *Blasdale* 5099 (UC); Wolf Creek, July 1916, *Abrams* 5857 (DS);
Napa County: St. Helena, Napa River, May 1897, *Jepson* (DS); near Calistoga;
April 1894, *Jepson* (UC); Santa Clara County: near Gilroy, 1882, *Palache*
(UC); Santa Cruz County: Big Basin, September 1901, *Dudley* (DS); by San
Lorenzo road, May 1893, *Dudley* (DS); Sonoma County: Guerneville, May
1903, *Heller* 6614 (DS); Trinity County: between Mad and Trinity Rivers on
Eureka–Red Bluff road, July 1916, *Abrams* 6199 (DS).

13. LATHYRUS LANSZWERTII Kellogg, Proc. Calif. Acad. 2:150.
1863.—*L. palustris* S. Wats., Bot. King's Exp. 79. 1871; *L. coria-
ceus* White, Bull. Torr. Bot. Club 21:452. 1894; *L. oregonensis*
White, Bull. Torr. Bot. Club 21:456. 1894, in part; *L. Goldsteinae*
Eastw., Bull. Torr. Bot. Club 32:197. 1905.—Perennial, herbage
slightly puberulent throughout; stems stout and climbing, 20–45 cm.
long, usually not branching; stipules slender, curved, lanceolate,
long acuminate, semi-sagittate, sometimes one-half size of adjacent
leaflets; leaflets 6–12, linear-lanceolate to elliptical, acute or obtuse,
very firm and coriaceous, strongly venulose, 3–5 cm. long; tendrils
well developed, commonly branching; peduncles rather slender, not
so long as leaves, bearing 4–10 purplish white flowers; flowers 10–25
mm. long; calyx teeth unequal, shorter than tube; pods glabrous,
3–4 cm. long; seeds few.—Fig. 5.

Washington, Oregon, Nevada, California to Arizona; Upper Sonoran Zone.
June–July. Type locality, Washoe, Nevada.

It seems evident[6] from a careful study of the original description of *L.
Lanszwertii* and the illustration which accompanies it, that WHITE must have
misunderstood this species, when he made it a synonym of *L. palustris* L. This
is made even more evident when specimens from the type locality are exam-
ined. *L. Lanszwertii* represents the same sort of plant as *L. Goldsteinae*, which
came from a similar region; both being practically the same as *L. coriaceus*,
except that that is a form with somewhat wider leaflets.

WASHINGTON.—Chelan County: Lake Wenache, August 1893, *Leiberg*
634 (UO, CA, UC); Wenache Forest, Old Baldy Mountain, August 1916,
*Eggleston* (US); Kittitas County: northeast Kittitas Valley, June 1903, *Cotton*
1208 (US); Yakima County: Yakima, June 18—, *Leckenby* (US).

[6] This was written before I had read HELLER's conclusion regarding the identity
of *L. Lanszwertii* (Muhlenbergia 6:92. 1910).

OREGON.—Baker County: Pine Creek and North Pine Creek, May 1886, *Cusick* 1372 (UO, US, UC); Pine Valley near Snake River, June 1898, *Cusick* 1918 (UO, UC); Crook County: Wolf Creek, Blue Mountains, July 1901, *Cusick* 2636 (UO, UC); Grant County: John Day River at Prairie City, July 1919, *Ferris* and *Duthie* 694 (DS); Harney County: near Mann Lake, June 1896, *Leiberg* 2404 (UO, UC); Steins Mountains, Divine Creek, July 1898, *Cusick* 1992a (UO, UC); Steins Mountains, July 1898, *Cusick* 1992 (DS, UO); Morrow County: 13.5 miles east of Heppener, July 1917, *Lawrence* 993 (DS).

CALIFORNIA.—El Dorado County: Fallen Leaf Lake, July 1906, *Thompson* (DS, US); Tallac, Lake Tahoe, July 1906, *Grant* (DS); Lakeside Park, Tallac, July 1908, *Brandegee* (UC); Tallac, July 1906, *Eastwood* 886 (CA); Camp Agassiz, July–August 1906, *Eastwood* 952 (CA); Plumas County: Quincy, July 1876, Mrs. *Austin* 29 (UC).

13a. **Lathyrus Lanszwertii aridus** (Piper), n. comb.—*L. oregonensis* White, Bull. Torr. Bot. Club **21**:456. 1894, in part; *L. graminifolius* of various authors, not Watson or White; *L. coriaceus aridus* Piper, Proc. Biol. Soc. Wash. **31**:190. 1918.—This subspecies has linear-lanceolate leaflets, 4–7 cm. long, and minute tendrils; flowers small, about 10 mm. long.—Fig. 6.

Washington, Oregon, and California; Upper Sonoran Zone. June–July. Type locality, "About Black Butte, Crook County, Oregon."

WASHINGTON.—Klickitat County: Falcon Valley, April 1885, *Suksdorf* (US).

OREGON.—Crook County: Black Butte, June 1902, *Cusick* 2814 (UO, DS, US); Farewell Bend, July 1894, *Leiberg* 433 (UC); Klamath County: near Lake of the Woods, July 1897, *Coville* and *Applegate* (US); Wasco County: White River, June 1881, *Howell* (DS); 3.5 miles west of Friend, June 1917, *Lawrence* 157 (DS).

CALIFORNIA.—Calaveras County: Bigtrees, Stanislaus Forest, June 1913, *Eggleston* 9212 (US); Plumas County: Quincy, June 1913, *Heller* 10851 (DS); Willow Creek, June 1912, *Hall* 9266 (UC); Tuolumne County: locality not given, June 1897, *Congdon* (UC); Yosemite, June 1911, *Hall* 8883 (DS).

14. **LATHYRUS PAUCIFLORUS** Fernald, Bot. Gaz. **19**:335. 1894.— *L. polyphyllus* S. Wats., Bot. King's Exp. 78. 1871; *L. ecirrhosus* Heller, Muhlenbergia **1**:54. 1904; *L. Bradfeldianus* A. Nels., Bot. Gaz. **54**:411. 1912.—Glabrous, frequently glaucous; stems stout, simple or branching, about 50 cm. high, climbing or erect; stipules large, usually half size of adjacent leaflets, broadly lanceolate, hastate; leaflets 6–12, elliptic to ovate-lanceolate, acute, usually firm and coriaceous, 1–4 cm. long; tendrils well developed or in certain

plants rather small and inconspicuous; peduncles either not so long
as leaves or longer, bearing 3–8 purple flowers; flowers 18–20 mm.
long; calyx glabrous, teeth unequal, upper triangular, 1–2 mm. long,
lateral linear-lanceolate, scarcely as long as tube, and lowest linear,
not exceeding tube; pods glabrous, 4 cm. long; seeds about 6.—Fig. 8.
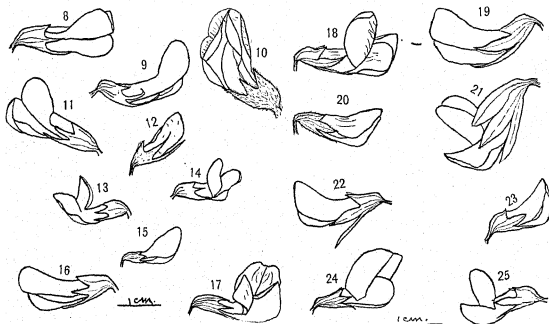


FIGS. 8–25.—Fig. 8, L. *pauciflorus*; fig. 9, L. *pauciflorus utahensis*; fig. 10, L.
*littoralis*; fig. 11, L. *pauciflorus tenuior*; fig. 12, L. *Torreyi*; fig. 13, L. *pauciflorus Brownii*;
fig. 14, L. *pauciflorus Schaffneri*; fig. 15, L. *bijugatus*; fig. 16, L. *polyphyllus*; fig. 17,
L. *Cusickii*; fig. 18, L. *Bolanderi violaceus*; fig. 19, L. *Bolanderi*; fig. 20, L. *Bolanderi*
*quercetorum*; fig. 21, L. *maritimus*; fig. 22, L. *ochropetalus*; fig. 23, L. *sulphureus*; fig. 24,
L. *rigidus*; fig. 25, L. *sulphureus*.

Washington, Oregon, Idaho, Wyoming, and California; Upper Sonoran
and Arid Transition Zones. April–July. Type locality, Almota, Washington.
On the whole I agree with PIPER's treatment of L. *pauciflorus* in "Some
Western Species of *Lathyrus*," and so his key to the subspecies has been used.
I do not think, however, that all the subspecies are of the same subspecific
value; but not having had field experience with them, I am not able from the
insufficient material examined to come to any further decisions. More field
work is needed to determine the exact distribution of the species and subspecies.
The species most closely allied to this group are found in the region dealt with in
RYDBERG's latest *Flora*. L. *pauciflorus* and its subspecies are similar in some
respects to L. *Lanszwertii*, but the latter is nearly always furnished with
puberulent herbage. L. *pauciflorus* is markedly glabrous and often glaucous.
The stipules in the L. *pauciflorus* group are usually enlarged, unless the plants
have narrow leaflets.

*L. pauciflorus Brownii* has often been confused with *L. graminifolius*, which apparently does not grow in California; at least I have seen no specimens. It is interesting to note here that *L. Lanszwertii aridus* has been mistaken for *L. graminifolius*. There is, it is true, a very superficial resemblance between *L. Lanszwertii aridus* and *L. pauciflorus Brownii*, and certain specimens collected by HELLER at Bennet Spring, Glenn County, California, seem to be somewhat intermediate between them.

*L. pauciflorus* has been confused with *L. polyphyllus*. One specimen collected by ALLEN in the upper valley of the Nesqually, in the Cascade Mountains, has a close resemblance to *L. polyphyllus*. *L. pauciflorus* has larger and fewer flowers; *L. polyphyllus* has more numerous leaflets, and they are never coriaceous. A specimen of *L. pauciflorus* with thin leaflets and numerous flowers suggests *L. polyphyllus* in general appearance, but the individual flowers are always different, the calyx teeth of the latter being ciliate.

There seems to be no apparent difference between *L. ecirrhosus* and *L. pauciflorus*.

WASHINGTON.—County not determined: upper valley of the Nesqually, Cascade Mountains, June–July 1895, *Allen* 132 (US, DS); Chelan County: between Drury and Chiwaukum, May 1920, *Otis* 889 (US); Hoose Lake region, near Wenache, May 1899, *Whited* 1106 (DS); hills across river from Leavensworth, May 1918, *Otis* 681 (CA); Klickitat County: hillsides near Columbia River, April–June 1886, *Suksdorf* 854 (US); Whitman County: Almota, June 1894, *Piper* (UC).

OREGON.—Counties not determined: Blue Mountains, May 1885, *Howell* (US); eastern Oregon, June 1881, *Howell* (UO); Clackamas County: Oswego, May 1891, *Gorman* (UO); Wallowa County: Fence Creek, no date, *Cusick* (UO); Lost Prairie, June 1900 (UO).

CALIFORNIA.—Lake County: Mt. Sanhedrin, July 1902, *Heller* 5944 (DS); Nevada County: Sugar Loaf Hill, Nevada City, June 1893, *Dudley* (DS); Plumas County: near Quincy, June 1913, *Heller* 10833 (DS); Sutter County: north side of Marysville Buttes, May 1914, *Heller* 11370a (DS).

14a. LATHYRUS PAUCIFLORUS UTAHENSIS (Jones) Piper, Proc. Biol. Soc. Wash. 31:194. 1918.—*L. utahensis* Jones, Proc. Calif. Acad. Sci. N. S. 2:678. 1895.—Distinguished from the species by the obtuse, oval to ovate leaflets.—Fig. 9.

Washington, Oregon, Utah, Colorado, and Arizona; Upper Sonoran and Arid Transition Zones. April–July. Type locality, "Ireland's Ranch, Utah, at the head of Salina Canyon."

OREGON.—Counties and localities not determined: 1884, *Cusick* (US 42779); *Cusick* (UO 1756. 1); Baker County: mountains near North Pine Creek, May 1901, *Cusick* 2538 (US, UO); Union County: locality not given, 1884, *Cusick* 756 (US).

14b. LATHYRUS PAUCIFLORUS TENUIOR Piper, Contrib. U.S. Nat. Herb. 11:378. 1906.—*L. parvifolius tenuior* Piper, Fl. Palouse Reg. 108. 1901; *L. tenuior* Rydb., Fl. Rocky Mts. 528. 1917.—This subspecies has linear-lanceolate leaflets, 3–6 cm. long.—Fig. 11.

Eastern Washington and Idaho; Arid Transition Zone. May–June. Type locality, "Snake River bluffs near Almota," Washington.

WASHINGTON.—Whitman County: Almota, 1896, *Elmer 52* (US).

OREGON.—Morrow County: near Rock Creek, May 1894, *Leiberg 77* (UC); Ochoco Nat. Forest, May 1912, *Ingram* (US); Wallowa County; locality not given, June 1900, *Cusick 2408* (US).

14c. LATHYRUS PAUCIFLORUS SCHAFFNERI Piper, Proc. Biol. Soc. Wash. 31:194. 1918.—*L. parvifolius* S. Wats., Proc. Amer. Acad. 17:345. 1882; *L. Schaffneri* Rydb., Mem. N.Y. Bot. Gard. 1:258. 1900.—This subspecies has flowers 12–16 mm. long, and oval, elliptic, or ovate leaflets; the tendrils are either well developed or minute.—Fig. 14.

Mexico, Arizona, New Mexico, Colorado, California, and Lower California; Upper Sonoran and Arid Transition Zones. April–July. Type locality, San Miguelito Mts., San Luis Potosi, Mexico.

CALIFORNIA.—County not determined: Sierra Nevada Mts., 1875, *Lemmon* (US); Butte County: Los Vergils, April 1920, *Kelley* (CA); Kern County: Water Canyon, Techachapi Mts., June 1908, *Abrams* and *McGregor 443* (DS); Modoc County: locality not given, June 1893, *Baker* (UC); Forestdale, 1893, *Baker* (UC); near Parker Creek, June 1919, *Ferris* and *Duthie 124* (DS); Plumas County: Massack Creek, May 1919, *Wagner 258b* (DS); Big Meadows, July 1878, Mrs. *Austin 39* (UC); Portola, May 1918, *Eastwood 7029* (CA); Sierra County: Sierra Valley, *Lemmon* (DS); Siskiyou County: locality not determined, April 1910, *Butler 1212* (DS).

14d. LATHYRUS PAUCIFLORUS BROWNII (Eastw.) Piper, Proc. Biol. Soc. Wash. 31:195. 1918.—*L. Brownii* Eastw., Bull. Torr. Bot. Club 30:491. 1903; *L. graminifolius* of various botanists, not Watson or White.—This subspecies has flowers about 10 mm. long, and linear or linear-lanceolate leaflets; the tendrils are frequently minute or absent.—Fig. 13.

Oregon, California, and Arizona; Arid Transition and Upper Sonoran Zones. May–July. Type locality, "North side of Mount Shasta."

OREGON.—Klamath County: June 1896, *Applegate 147* (US).

CALIFORNIA.—Glenn County: west of Bennet Spring, June 1915, *Heller 12006*; this is somewhat unlike typical *L. pauciflorus Brownii* (CA); Kern

County: Water Canyon, Techachapi Mts., June 1908, *Abrams* and *McGregor*
443 (US, DS. Some of the sheets with this number have specimens of *L. pauci-
florus Brownii*, while others have *L. pauciflorus Schaffneri* and variations be-
tween the two); Lassen County: Susanville, July 1892, *Brandegee* (UC); Nevada
County: Castle Crag trail, June 1893, *Dudley* (DS); Plumas County: Massack
Creek, May 1919, *Wagner 258a* (DS); Prattville, summer 1906, Mrs. *Coombs*
(CA); Shasta County: Burney, June 1912, *Eastwood* (CA); Castilla, May 1904,
*Piper* 6367 (US); Sierra County: Portola, May 1918, *Eastwood* (CA); Siskiyou
County: Weed, May 1913, *Smith* 272 (CA. Flowers large, suggesting the sub-
species *tenuior*); north side of Mount Shasta, June 1897, *Brown* (US. Duplicate
type); Mt. Eddy, July 1915, *Heller* 12996 (DS); Greenhorn Mountain, May
1910, *Butler* 1346 (DS); Tehama County: Red Bluff, June 1917, *Wicks* (CA).

15. LATHYRUS POLYPHYLLUS Nutt. T. and G., Fl. N.A. 1:274.
1838.—Perennial, glabrous throughout; stems erect or nearly so,
climbing, stout and angled, 70–100 cm. high; stipules as large as
adjacent leaflets, semicordate, frequently dentate; leaflets 10–20, dis-
tinctly petiolulate, paler beneath, somewhat flaccid, oblong, acute
or obtuse, 2–4 cm. long; tendrils well developed; peduncles slender,
not so long as leaves; flowers 6–10, purple, 15–20 mm. long; calyx
teeth ciliate, upper 2 triangular, others subulate, as long as tube;
pods linear-oblong, glabrous, 5 cm. long; seeds 2–10.—Fig. 16.

Common in open coniferous woods from British Columbia to northern
California; Humid Transition Zone. May–August. Type locality, "Forests of
the Oregon toward the sea."

WASHINGTON.—Clallam County: Olympic Mountains, August 1900, *Elmer*
2535 (DS); King County: Mercer Island, Seattle, June 1920, *Eastwood* 9956
(CA); Klickitat County: locality not given, May 1891, *Suksdorf* 2021 (DS);
Pierce County: Tacoma, June 1920, *Eastwood* 9642 (CA).

OREGON.—Douglas County: Rogue River, near Elk Creek, July 1899,
*Leiberg* (UO); Lane County: Eugene, May 1920, *Bradshaw* 1555 (RVB); Marion
County: Salem, April 1915, *Nelson* 69 (DS); Multnomah County: Rocky Butte,
May 1903, *Sheldon* 12032 (DS, UO); Portland, May–June 1883, *Henderson*
(DS, UO).

CALIFORNIA.—North Coast Ranges: Bell Springs to Harrison, May–
August 1899, *Davy* and *Blasdale* 5355 (DS, UC); data not given: State Survey
4685 (UC); Humboldt County: Valley of Van Duzen River, opposite Buck Mt.,
June–July 1908, *Tracy* 2731 (UC); Little Bear Harbor, Geol. Surv. Calif. 6508,
*Bolander* (UC); Mendocino County: Idol House, May 1901, *Chandler* 1075 (UC);
Sherwood, May 1899, *Davy* and *Blasdale* 1068 (UC); Trinity County: Trinity
Summit, July 1901, Mrs. *Manning* 62½ (UC).

16. LATHYRUS BOLANDERI S. Wats., Proc. Amer. Acad. 20:363.
1885.—Stems stout, up to meter in height, herbage glabrous or
puberulent, perennial; stipules half as large as adjacent leaflets,
dilated, semi-sagittate, often toothed; leaflets 6–10, lanceolate to
broadly ovate, acute or obtuse, usually thin but sometimes coria-
ceous, 25–50 mm. long; tendrils stout, well developed and branching;
peduncles equaling or exceeding leaves, bearing 8–10 flowers, from
blue to white or pink in color, each about 20 mm. long; calyx teeth
unequal, upper 2 about 2 mm. long, lateral linear-lanceolate 8 mm.
long, lowest linear often 10 mm. long, and frequently twice the length
of calyx tube; pods glabrous, 5–6 cm. long; seeds 3–6.—Fig. 19.

Southern Oregon to southern California along the coast; Humid Transition
and Upper Sonoran Zones. March–June. Type locality, "Oakland Hills near
San Francisco."

This is one of the most abundant species on the western or seaward slope
of California. Its most striking features are its enlarged stipules and broad,
elongated calyx teeth. The pubescence is very variable. The difference between
this species and what has been known as *L. violaceus* or *L. vestitus puberulus* is
very slight. The essential characters are the same, such as the characteristic
calyx teeth and the general habit. As has been mentioned before, the stipules
and leaflets are extremely inconstant in shape and size. At best I should con-
sider *L. quercetorum* but a subspecies of *L. Bolanderi*. The latter often has tawny
flowers. The pubescence of the former is probably its best and most pronounced
character. What the original *L. vestitus* was, which NUTTALL collected from the
"Columbia plains toward the sea," no botanist has been able to determine.
Possibly it was something similar to *L. Bolanderi*, or *L. Bolanderi violaceus*.
It apparently has never been collected by any one since that time.

OREGON.—Douglas County: Glendale, June 1902, *Jones* (US).

CALIFORNIA.[7]—Alameda County: Point Isabel, West Berkeley, April 1897,
*Davy* (UC); Contra Costa County: Fish Ranch, *Jepson* (UC); Del Norte
County: Smith River, April 1902, *Goddard* 328 (UC); Humboldt County:
Willow Creek, June 1918, *Abrams* (DS); Marin County: Mt. Tamalpais,
November 1914, *Eastwood* (CA); Mendocino County: Kaisen District, June
1903, *McMurphy* 132 (DS); Monterey County: between Pebble Beach and
Carmel, March 1910, *Randall* 167 (DS); Napa County: Mt. St. Helena, March
1907, collector not given (CA); San Francisco County: south end of Lake Mer-
ced, May 1901, *Dudley* (DS); San Mateo County: Crystal Lake, April 1902,
*Abrams* 2342 (DS); Sonoma County: Duncan's Mills, July 1882, *Jones* 3576
(CA); Ventura County: San Buenaventura, Geol. Survey, March 1861, *Brewer*
236 (US).

[7] Space does not permit a citation of all the specimens examined of the species
and the subspecies *violaceus*.

16a. **Lathyrus Bolanderi quercetorum** (Heller), n. comb.—*L. quercetorum* Heller, Muhlenbergia 2:290. 1907.—This subspecies is more dwarfed than the species; the leaflets are covered with appressed white hairs; the flowers are tawny, and the calyx teeth not so long as with the species, but are similar in shape.—Fig. 20.

Mt. Hamilton and Mt. Diablo, California; Upper Sonoran Zone. April. Type locality, "Near the summit of Mt. Hamilton, Santa Clara County," California.

CALIFORNIA.—Napa County: Mt. Diablo, April 1862, State Survey (UC); Santa Clara County: Mt. Day in the Hamilton Range, April 1908, *Heller* 8926 (DS); Mt. Hamilton, May 1907, *Heller* 8623 (DS); same locality, 1891, *Greene* (UC).

16b. **Lathyrus Bolanderi violaceus** (Greene), n. comb.—*L. violaceus* Greene, Erythea 1:105. 1893; *L. puberulus* White; Greene, Man. 85. 1894; *L. vestitus puberulus* Jepson, Fl. W. Mid. Cal. 1: 298. 1901.—This subspecies is more puberulent than the species; the stipules more narrow, smaller, and the flowers less numerous; the leaves are often darker, more coriaceous, and the stems longer and more branched, and more frequently found climbing over bushes than the smaller more succulent typical *L. Bolanderi;* somewhat more like *L. Bolanderi quercetorum*, although all sorts of variations occur.—Fig. 18.

Northern to southern California in the coast region; Upper Sonoran and Humid Transition Zones. March–June, often flowering in the late fall or winter. Type locality, type specimens grown at the University of California from seeds collected in the mountains of Los Angeles County, California.

WHITE's *L. violaceus barberae* from the description seems to be one of the many variations of *L. Bolanderi*. These leaflet variations are anything but constant; however, I have seen no material so designated by WHITE. He described the calyx thus: "upper calyx teeth exceeding the tube, lateral ones broader and about the length of the tube, the lowest broad and much shorter." Part of this must be a misprint, for "upper calyx teeth exceeding the tube" is certainly very far from true with any of the plants related to *L. Bolanderi*, although the statement regarding the lateral teeth agrees with most of the material of *L. Bolanderi* and its allies. It is interesting to note that in the material examined in the various herbaria, nearly all the collectors have used the name *L. violaceus barberae* for forms of *L. laetiflorus Alefeldi* with narrow leaflets. It is clear, however, that the description of the form in question suggests *L. violaceus* of GREENE, rather than any of the species related to *L. splendens*, which never have broad or greatly elongated calyx teeth.

WHITE considered that *L. strictus* Nuttall (not *L. strictus* Grauer, 1784) might have been either *L. violaceus* or *L. violaceus barberae*, or even both. This may be true, but the possibility is that NUTTALL's *strictus* was a form of *L. laetiflorus Alefeldi* with very narrow leaflets, for all those allied to *L. splendens* have occasionally narrow leaflets, especially in the upper part of the plant.

The following specimens are referred to *L. Bolanderi violaceus.*

CALIFORNIA.—Alameda County: Oakland, May 1887, *Rattan* (DS); U. of C. Botanic Gardens, 1893, *Davy* (UC. From the type); Contra Costa County: Camp 69, Walnut Creek, April 1862, *Brewer* 1018 (UC); Lake County: Sulphur Banks, April 1902, *Bowman* 168 (DS); Los Angeles County: Santa Monica Mts., April 1901, *Abrams* (DS); Marin County: Corte Madera, March 1903, *Sheldon* 11564 (UO); Monterey County: Pacific Grove, April 1909, *Abrams* 4208 (DS); Napa County: St. Helena, July 1891, *Greene* (UC); Santa Barbara County: Santa Inez River, May 1907, *Hall* 7826 (UC); Santa Clara County: foothills west of Los Gatos, April 1904, *Heller* 7283 (DS); Santa Cruz County: Santa Cruz, June 1903, *Thompson* (DS); San Francisco County: Lake Merced, May 1918, *Eastwood* (CA); San Luis Obispo County: San Luis Obispo, spring 1905, *Roadhouse* 87 (UC); San Mateo County: Crystal Springs Lake, May 1922, *Bradshaw* 2752 (RVB); Sonoma County: near Sonoma, April 1862, *Brewer* 966 (UC); Ventura County: Saticoy, April 1916, *Eastwood* 5049 (CA).

17. LATHYRUS JEPSONII Greene, Pittonia 2:158. 1890.—*L. palustris* L. var. ε T. and G., Fl. N.A. 1:276. 1838.—Perennial, nearly glabrous; stems with prominent wings, 1.5–3 m. high, rather stout; stipules much smaller than adjacent leaflets, semi-sagittate, acuminate, entire or toothed; leaflets 8–12, linear-lanceolate, acute, usually firm and coriaceous, veins very pronounced, 3–5 cm. long; tendrils well developed, commonly divided; peduncles stout, 15–30 cm. long, about equaling leaves; flowers 6–15, usually 20 mm. long, rose-purple, becoming darker upon fading and drying; calyx teeth unequal, 2 upper very short, other 3 about as long as tube; pods glabrous, 7 cm. long, compressed; seeds 12–16.—Fig. 4.

Tidewater sloughs at Suisun, California, also at Stockton; Upper Sonoran Zone. August–September. Type locality, "Suisun marshes, also toward Stockton."

*L. Jepsonii* is a very local species, doubtless closely allied to *L. Watsonii*, and both suggest relationship with certain forms of *L. Bolanderi* rather than with *L. palustris* L. This is shown by the large flowers of *L. Jepsonii* and *L. Watsonii*, as well as by the size and shape of the calyx teeth. The calyx teeth of *L. Jepsonii* and *L. Watsonii* are nearly always shorter than those of *L. Bolanderi*, but although the calyx teeth are shorter, they are in both cases of similar shape. If *L. palustris* is related to any of the American species of *Lathyrus*, it is probably

closest to *L. Lanszwertii*, but the relationship is distant, for *L. palustris* is distinct. While *L. palustris* and *L. Jepsonii* are most commonly found near water, *L. Watsonii* is frequently collected in drier habitats.

The following specimens are referred to *L. Jepsonii*.

CALIFORNIA.—Napa County: tidal marsh in Napa River, August 1892, *Bioletti* (DS); Drawbridge, August 1892, *Bioletti* (UC); San Joaquin County: Stockton, 1903, *Berg* (DS); Rough and Ready Island, November–December 1903, *Berg* (UC); Solano County: Suisun Marshes, October 1905, *Dudley* (DS); same locality, June 1903, *Baker 3226* (US, DS); same locality, August 1903, *Baker* (US); same locality, August 1920; *Jones* (CA); same locality, July 1913, *Eastwood 3445* (CA).

18. LATHYRUS WATSONII White, Bull. Torr. Bot. Club **21**:447. 1894.—*L. venosus californicus* S. Wats., Proc. Amer. Acad. **11**:133. 1876; *L. californicus* S. Wats., Proc. Amer. Acad. **20**:363. 1885.— Perennial, puberulent or velvety pubescent; stems ordinarily with prominent wings, 1–2 meters high, rather stout and frequently branching; stipules not half so large as the adjacent leaflets, semi-sagittate, dilated, commonly toothed at base, long acuminate; leaflets 8–12, ovate to linear-lanceolate, obtuse or acute, very firm and coriaceous, strongly venulose, 1–5 cm. long; tendrils well developed, divided; peduncles stout, 10–25 cm. long, not exceeding leaves, bearing 6–15 flowers; flowers about 20 mm. long, white veined with pink or purple, fading to deep yellow; calyx teeth unequal, upper 2 very short, 3 lowest scarcely exceeding tube; pods glabrous, 5–6 cm. long; seeds 4–6.—Fig. 1.

California as far south as Monterey County; Upper Sonoran and Arid Transition Zones. April–July. Type locality, Monterey and Sonoma Counties.

CALIFORNIA.[8]—Alameda County: Alameda, May 1891, *Greene* (DS); Amador County: Panther Creek, 1895, *Hansen* 1299 (DS); Butte County: Berry Canyon, near Clear Creek, May 1902, *Heller* and *Brown* 5489 (DS); Calaveras County: Murphy's Camp, May 1895, *Davy* 1492 (UC); Contra Costa County: Alhambra Valley, May 1887, *Rattan* (DS); El Dorado County: Pyramid Peak, 1900, *Atkinson* (DS); Fresno County: Toll House, June 1900, *Hall* and *Chandler* 37 (UC); Humboldt County: Dinsmore's Ranch, valley of Van Duzen River, June 1913, *Tracy* 4110 (UC); Kern County: Bisse's Station, June 1895, *Dudley* 485 (DS); Lake County: Mt. Sanhedrin, July 1913, *Hall* 9494 (UC); Marin County: White's Hill, August 1920, *Eastwood* 10016 (CA); Mariposa County: locality not given, April 1915, *Eastwood* 4359 (CA); Mendocino County: Sherwood Valley, June 1899, *Dudley* (DS); Monterey County:

[8] A greatly abbreviated list of specimens examined.

Castroville, May–June 1901, *Davy* 7546 (DS, UC); Napa County: along Napa
River near Ritherford, May 1904, *Tracy* 2079 (UC); Placer County: Emigrant
Gap, July 1882, *Jones* 3573 (CA); Plumas County: thickets along streams,
July 1900, *Leiberg* 5180 (UO); San Benito County: Hernandez, June 1903;
*Lathrop* (DS); Santa Clara County: foothills near Stanford University, May
1902, *Baker* 849 (CA, UC); Santa Cruz County: locality not given, June 1914,
*Smith* 2891 (CA); San Mateo County: Crystal Spring's Lake, May 1922, *Brad-
shaw* 2753 (RVB); San Luis Obispo County: Paso Robles, May 1899, *Barber*
(UC); Shasta County: Redding, May 1913, *Smith* 238 (CA); Sierra Nat.
Forest: July 1912, *Abrams* 4979 (DS); Sierra County: Cedar Glen, May 1920,
*Jones* (CA); Siskiyou County: by Sacramento River, August 1905, *Dudley*
(DS); Solano County: Elmira, May 1903, *Baker* 2922 (UC, CA); Sonoma
County: Santa Rosa Creek, east of Santa Rosa, June 1902, *Heller* 5659 (DS);
Trinity County: Coffee Creek, Salmon Mts., July 1909, *Hall* 8527 (UC); Tulare
County: Kaweah River, 18 miles east of Visalia, March 1898, *Woolsey* (UC).

19. LATHYRUS PALUSTRIS L., Sp. Pl. 733. 1753.—*L. occidentalis*
Nutt.; T. and G., Fl. 1:276. 1838, as a synonym.—Perennial,
glabrous or pubescent; stems slender, climbing, about 60 cm. high,
wings often as broad as stem; stipules small or sometimes one-third
size of adjacent leaflets, narrow, acuminate, semi-sagittate; leaflets
4–6 (or rarely 2), firm, acute or obtuse, linear to oblong, 2–5 cm. long;
tendrils well developed; peduncles longer than leaves, bearing 2–6
purple flowers; flowers 10–30 mm. long; calyx teeth very unequal,
2 upper scarcely more than 1–2 mm. long, 3 lower nearly as long as
tube; pods glabrous, linear oblong, 5 cm. long; seeds 6–8.—Fig. 2.

In marshes especially along the seashore; Humid Transition Zone; Alaska to
northern California, New England, and Europe. May–August. Type locality,
"In Europe borealis pascuis paludosis."

OREGON.—Clatsop County: Waluski River, August 1902, *Sheldon* 10220
(DS, UO); Douglas County: mouth of Umpqua River, June 1885, *Howell*
(DS); Lincoln County: Newport, July 1919, *Bradshaw* 712 (RVB); 9 miles
south of Newport, June 1918, *Lawrence* 1783 (US).

CALIFORNIA.—Butte County: De Sabla, June 1917, *Edwards* (DS 81643,
UO. Flowers large; leaflets 2); Del Norte County: Lake Earle, June 1902,
*Davy* (UC); Humboldt County: near Samoa, July 1907, *Tracy* 2593 (DS, UC);
Mendocino County: barrens back of Mendocino, June 1903, *McMurphy* 473
(DS).

20. LATHYRUS MARITIMUS (L.) Bigel., Fl. Bost. ed. 2. 268.
1824.—*Pisum maritimum* L., Sp. Pl. 727. 1753; *L. californicus*
Dougl.; Lindl., Bot. Reg. *pl.* 1144. 1828; *L. pisiformis* Hook., Fl.

Bor. Am. 1:158. 1834.—Perennial, glabrous, slightly glaucous; stems stout, decumbent, 25–90 cm. long; stipules large, about half size of adjacent leaflets, ovate, acute, entire or lower lobe dentate; leaflets 8–12, pale and rather fleshy, nearly sessile, oblong to ovate, acute or obtuse, 1–5 cm. long; tendrils well developed; peduncles about length of leaves; flowers 6–10, purple and white, nearly 20 mm. long; calyx teeth unequal, 2 upper as long as tube, 3 lower longer than tube; pods flat, slightly pubescent, 5–6 cm. long; seeds about 10.—Fig. 21.

   Along sea coasts, Alaska to California, shores of Great Lakes, Labrador to New Jersey, and northern Europe and Asia; Humid Transition Zone. May–August. Type locality, "Habitat in Europae borealis littoribus maris arenosis."

   WASHINGTON.—Camano Island, June 1896, *Gardner* (UC.); Chehalis County: near South Arbor, May 1897, *Lamb* 1112 (DS); Clallan County: Olympic Mts., near coast, *Elmer* 2528 (DS); La Push, June 1921, *Taylor* (CA); King County: Fort Lawton, Seattle, June 1920, *Eastwood* 9621 (CA); Pacific County: Ilwaco, July 1886, *Henderson* (DS, UO); San Juan County: Brown's Island, Friday Harbor, July 1904, *Berg* 61 (DS); False Bay, San Juan Island, June 1919, *Roush* (DS).

   OREGON.—Clatsop County: Clatsop Beach, July 1916, *Nelson* 822 (DS); Lincoln County: Newport, July 1919, *Bradshaw* 713 (RVB).

   CALIFORNIA.—Del Norte County: near Crescent City, October 1909, *Tracy* 3107 (UC); Humboldt County: Eureka, Humboldt Bay, July 1912, *Tracy* 3736 (UC).

   21. LATHYRUS OCHROPETALUS Piper, Proc. Biol. Soc. Wash. 31: 189. 1918.—Perennial, perfectly glabrous throughout and somewhat glaucous; stems climbing, slender, about 1–2 m. high; stipules large, half size of adjacent leaflets, ovate, nearly entire; leaflets 4–10, ovate to ovate-lanceolate, acute, membranaceous, paler beneath, 3–6 cm. long; tendrils well developed, branching; peduncles rather stout, about half length of leaves; flowers 7–13, pale yellow, 20 mm. long; calyx teeth unequal, 2 upper triangular, scarcely over 1 mm. long, lateral oblong-lanceolate, as long as tube, ventral slightly exceeding tube; pods glabrous, 4 cm. long; seeds about 6.—Fig. 22.

   Western Washington and Oregon; Humid Transition Zone. May–June. Type locality, Seattle, Washington.

   More field work will be required in order to determine the exact relationship and position of *L. ochropetalus*. From the material examined, it seems to be intermediate between *L. Bolanderi* and *L. sulphureus*, and suggests also a con-

nection with *L. polyphyllus*, especially in the shape of the leaflets and the peculi-
arity of the foliage. This species may prove to be only a northern form of *L.
Bolanderi*, however, the floral characters of these being very similar, particularly
the calyx teeth. *L. sulphureus* always has smaller flowers. In the herbaria *L.
ochropetalus* has passed for both *L. sulphureus* and *L. polyphyllus*. Much that
goes by the name of *L. sulphureus* is really only a yellow flowered form of *L.
Bolanderi*. The distribution of these is very different. *L. Bolanderi* is a plant of
the immediate region of the sea coast; where this species leaves off in the north,
*L. ochropetalus* appears to begin. *L. sulphureus*, however, although found in the
Humid Transition is more common in the Arid Transition, particularly so in
California, and sometimes extends into the Upper Sonoran, as at Auburn,
California. As has been mentioned, the calyx teeth of *L. Bolanderi* and *L.
ochropetalus* are very similar in shape and size, but *L. sulphureus* has very differ-
ent calyx teeth; they are shorter and not so broad. There are forms of *L.
sulphureus*, however, which, although having the small flowers which are so
characteristic, yet have calyx teeth strongly suggesting those of *L. Bolanderi*.
These forms may be hybrids, but at any rate show that these species are closely
allied. *L. ochropetalus holochlorus* seems to be only a form of *L. sulphureus* with
very thin leaflets. The specimen cited by PIPER does have thin leaflets, but
another specimen collected in 1918 by LAWRENCE from a similar locality has the
coriaceous leaflets of *L. sulphureus*.

The following material of *L. ochropetalus* has been examined:

WASHINGTON.—King County: Seattle, 1891, *Piper 482* (US 218905 and
202210); same locality, June 1892, *Mosier* (US).

OREGON.—Coos County: Myrtle Point, June 1893, *Holzinger* (US); Curry
County: Gold Beach, *Hoyt* (RVB); Jackson County: Antelope Creek, June
1898, *Applegate 2361* (US).

22. LATHYRUS SULPHUREUS Brewer; A. Gray, Proc. Amer.
Acad. 7:399. 1867.—*L. ochroleucus* var. Torr., Pac. R. R. Rep. 4:77.
1857; *L. ochroleucus* of various authors, not Hooker; *L. ochropetalus
holochlorus* Piper, Proc. Biol. Soc. Wash. 31:190. 1918.—Perennial,
nearly glabrous and somewhat glaucous; stems slender, climbing,
about a meter long; stipules half as large as adjacent leaflets,
coriaceous, semi-sagittate, entire or dentate; leaflets 8 or more,
ovate-lanceolate to ovate, acute, coriaceous, light green on both
surfaces; tendrils well developed, usually branching; peduncles
shorter than or as long as leaves, bearing 7–19 yellowish flowers;
flowers 10–12 mm. long; calyx teeth unequal, upper 2 very short,
triangular, lateral linear-lanceolate, scarcely as long as tube, ventral
tooth narrowly linear, as long as or longer than tube; pods 6 cm.
long, glabrous; seeds 6–7.—Figs. 23, 25.

Western Washington and Oregon to southern California; Humid Transition, Arid Transition, and Upper Sonoran Zones. April–July. Type locality, "In woods along foothills of Sierra Nevada."

For many years *L. sulphureus* has been confused with *L. ochroleucus*, which is quite different. *L. ochroleucus* may grow in this region, and the one specimen I have listed of *L. sulphureus* from Washington is very close to it. The only specimens of the true *L. ochroleucus* that I have observed in the field were in Wisconsin. The leaflets of *L. ochroleucus* are thin and obtuse, usually very broad, and are nearly always in three pairs.

WASHINGTON.—Okanogan County: Loomis, June 1902, *Griffiths* and *Cotton* 337 (US).

OREGON.—Benton County: Corvallis, May 1916, *Gilbert* 115 (US); same locality, May 1918, *Lawrence* 1459 (US); Curry County: Brookings, May 1915, *Thompson* 206 (DS); Douglas County: Glendale, June 1896, *Howell* (DS); same locality, June 1895, *Howell* 160 (UC); Lane County: Eugene, April 1905, *Sweetser* (UO); Springfield, May 1920, *Bradshaw* 1451 (RVB); Marion County: Quinaby, April 1915, Nelson (DS); Washington County: Forest Grove, May 1883, collector not given (UO).

CALIFORNIA (a greatly abbreviated list).—Amador County: locality not given, May 1893, *Hansen* 12 (DS, CA); Butte County: Berry Canyon, near Clear Creek, May 1902, *Heller* and *Brown* 5485 (US); Calaveras County: Mokelumne Hill, *Blaisdell* (CA); Fresno County: Pine Ridge, June 1900, *Hall* and *Chandler* 287 (UC); Glenn County: locality not given, June 1915, *Heller* 11994 (DS); Kern County: Green Horn Mts., *Palmer* 47 (US, UC); Lake County: near Snow Mt., June 1891, *Brandegee* (UC); Los Angeles County: Mazana, Antelope Valley, May 1896, *Davy* 2463 (UC); Mariposa County: Benton Mills, April 1883, *Congdon* (DS); Nevada County: Comptche, June 1906, *Walker* 253 (UC); Placer County: Blue Canyon, June 1908, *Walker* 299 (UC); Plumas County: Greenville, August 1920, *Clemens* (CA); Shasta County: Kennet, May 1913, *Smith* 160 (CA); Siskiyou County: locality not given, May 1909, *Quinyly* 707 (UC); Sutter County: north side of Marysville Buttes, May 1914, *Heller* 11370a (DS); Tehama County: Red Bluff, June 1917, *Wickes* (CA); Trinity County: east Weaver Creek, May 1914, *Yates* 480 (US); Tuolumne County: near French Flat, April 1919, *Ferris* 1584 (DS); Yosemite: Eightmile, June 1911, *Hall* 8985 (UC).

23. LATHYRUS SPLENDENS Kellogg, Proc. Calif. Acad. 7:90. 1876.—*L. venosus grandiflorus* Torr. Pac. R. R. Rep. 77. 1857.— Perennial, glabrous to puberulent; stems climbing, 1–3 m. long; stipules much smaller than adjacent leaflets, lower lobes denticulate or laciniate, semi-sagittate, acute to acuminate, very veiny and coriaceous; leaflets 4–10, linear-lanceolate to ovate, firm and with prominent veins, about 2–5 cm. long; tendrils well developed; peduncles

stout, 5–20 cm. long, much exceeding leaves; flowers 5–12 in the raceme, purple or dark red, very showy, over 3 cm. long; calyx teeth unequal, upper teeth broadly triangular, lateral not quite so broad, lowest linear-lanceolate to subulate, none longer than calyx tube; pods glabrous, 9 or more cm. long by 1 cm. wide; seeds about 15.—Fig. 26.
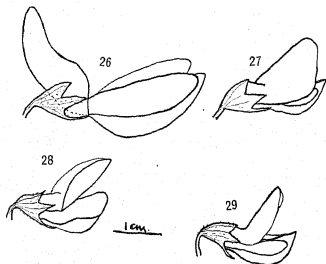
Southern California, Lower California, and Mexico; Upper Sonoran Zone. May–July. Type locality, southern California.

L. splendens, L. laetiflorus, and what has been known as L. Alefeldi constitute a very distinct group of American species of Lathyrus. The foliage of all of them is similar, and the structure of the calyx teeth and flowers indicates a decided relationship. The shape of the flowers is very much unlike that of our other species. When L. splendens is in full bloom, the standard pushes back so that it rests nearly in a straight line with the wings. Although L. splendens is quite distinct from the other members of this group, there are specimens that appear to be somewhat intermediate. Among these can be cited Miss EASTWOOD'S no. 9433. In general appearance this suggests L. splendens, but the flowers are entirely too small to be included with that species. L. Alefeldi and L. laetiflorus intergrade in such a way that at times they can scarcely be told apart; hence it seems desirable to give L. Alefeldi but subspecific rank, since the chief difference is only a matter of color.

A vast amount of the material that has passed as L. violaceus from southern California is really what I should call L. laetiflorus Alefeldi. The latter has also been confused with L. splendens by many collectors. Some botanists speak of L. laetiflorus Alefeldi as the little flowered L. splendens. The distribution of these species is different, however; L. splendens is not found so far north.

The following specimens will give an idea of the California distribution of L. splendens.

Imperial County: Summit, June 1917, McGregor 957 (DS); San Diego County: Campo, June 1880, Parish 1638 (DS); Potrero, May 1893, Alderson (DS); Potrero Grade, June 1913, Hall 9410 (UC).

24. LATHYRUS LAETIFLORUS Greene, Erythea 1:105. 1893.— Perennial, glabrous or puberulent; stems stout and climbing, several



FIGS. 26–29.—Fig. 26, L. splendens; fig. 27, L. laetiflorus Alefeldi; fig. 28, L. laetiflorus; fig. 29, L. laetiflorus Alefeldi (form with narrow leaflets).

meters long, branching; stipules small, not half so large as adjacent leaflets, commonly toothed, acuminate; leaflets 5–10, oblong to ovate, obtuse or acute, very firm and coriaceous, strongly venulose, 1–5 cm. long; tendrils well developed, long and divided; peduncles rather stout, 10–20 cm. long, frequently twice length of leaves; flowers 5–12 in the raceme, white or pink, 20–25 mm. long; calyx teeth unequal, upper 2 very short, lateral and ventral as long as calyx tube or less, very variable as to shape; pods flattened, slightly pubescent; seeds 4–8.—Fig. 28.

Southern California and northern Lower California; Upper Sonoran Zone. January–July. Type locality, seeds collected in Los Angeles County were germinated, and the plants raised at the University of California.

CALIFORNIA.—Los Angeles County: Mt. Wilson, Sierra Madre trail, June 1914, *Smith* 2865 (CA); Claremont, March 1916, *Cox* (US); Pasadena, Arroyo Seco, April 1903, *Hall* 3742 (UC, UO); Lincoln Park, May 1902, *Grant* (DS); plants grown at Berkeley, from seeds collected in Los Angeles County, May 1893 (UC); Orange County: Santiago Peak, June 1901, *Abrams* 1813 (DS); Riverside County: near Riverside, 1889, *Hosp* (UC); Santa Barbara County: Santa Cruz Island, June 1918, Mrs. *Miller* (CA); San Bernardino County: San Bernardino Valley, April 1906, *Parish* 5589 (UO); San Diego County: Fall Brook, November 1891, *Parish* (UC); Ventura County: piñon belt, Mt. Pinos, June 1905, *Hall* 6428 (UC).

24a. **Lathyrus laetiflorus Alefeldi** (White), n. comb.—*Orobus californicus* Alef., Bonplandia **9**:146. 1861, excl. syn.; *L. Alefeldi* White, Bull. Torr. Bot. Club **21**:449. 1894.—This subspecies is similar to the species, save that the flowers are blue or purple, and slightly larger (2–3 cm. long), and the stipules more variable in size, frequently attaining one-half the size of the adjacent leaflets.—Figs. 27, 29.

Southern California, northern Lower California, and Santa Catalina Island; Upper Sonoran Zone. March–July. Type locality, San Diego, California.

CALIFORNIA.[9]—Los Angeles County: Avalon, Santa Catalina Island, April 1901 *Grant* (DS); Gawanza foothills, June 1903, *Palmer* (UC); San Fernando Valley, February 1861, *Brewer* 199 (CA); Soledad, April 1882, *Jones* 3575 (US, CA); Riverside County: San Jacinto Mts., June 1901, *Hall* 2153 (US); San Bernardino County: San Bernardino, May 1882 (UC 15350); San Diego County: Warner's Ranch, June 1913, *Hall* 9427 (UC); San Miguel Mountain, May 1904, *Chandler* 5274 (DS); Mission Hills, May 1903, *Abrams* 3417 (DS).

PALO ALTO, CALIFORNIA

9 Space does not permit the citation of the quantities of material of this abundant species and subspecies, or that of *L. splendens*, and the various other species which I have examined.

# BRITTLE RACES OF OENOTHERA LAMARCKIANA

## HUGO DE VRIES

Brittleness is a quality which belongs to the central group of characters in *Oenothera*, as distinguished by BOEDIJN and the writer (9). Like almost all the members of this class, it is not accompanied by the doubling of a chromosome. Moreover, it is recessive and isogamic, and therefore can be combined in the mutant races with any other mark. This article reports the study of its relations to the lethal factors, which are also situated in that group. This study starts from two races, one of which (*O. similis*) has been found in the original field at Hilversum, lately described by UPHOF (2), while the other (*O. scindens*) originated in my experimental garden at Amsterdam. These races are to be considered as due to recombinations of mutated qualities, the first origin or premutation of which is probably as old as the parent species itself. Secondary changes may accompany them, but they are not of paramount interest for the present study.

### Oenothera Lamarckiana similis

In crosses of individuals of the same family of *O. rubrinervis* with *Lamarckiana* plants from various sources, and with different mutant strains, I observed that in the first generation sometimes brittle *rubrinervis* are produced, but in other cases tough hybrids of the type of *subrobusta* instead of *rubrinervis* (3). From a single cross between two individual parents both types are never seen to arise in the first generation. Moreover, the two contrasting cases have occurred mainly in strains derived from different initial plants (3). *Rubrinervis* is only a brittle form of *subrobusta*, and this latter usually splits off brittle specimens in the second generation. On the other hand, we have to consider *O. erythrina* (5), which is a mutant externally very like the hybrid *subrobusta*. It never arose by way of mutation from the main strain of *O. Lamarckiana*, which I am still using for my cultures, and which produces regularly brittle mutants of the type of *rubrinervis*. From rosettes collected at the original station near Hilversum in 1905, however, it sprung in one instance

in two specimens in 1907, and from another rosette in three individuals in the third generation in 1917. From one of the two first ones my family of *erythrina* has been derived.

Those initial rosettes of 1905 which produced *erythrina* mutants instead of brittle ones, might be considered as constituting a special mutant race, sprung in the field from the ordinary *Lamarckiana*. Of one of them seeds were no longer available, but in the third generation derived from the other in 1917, I have made some crosses, besides self-fertilizing some flowers in order to continue the race. I shall call this race *O. Lamarckiana* mut. *similis*, since it is externally an exact copy of its parent species, differing from this in no visible character and at no single period of its development. Now the question arose, whether the production of tough or brittle hybrids is connected with that of throwing off tough or brittle mutants. If this were so, we should expect the normal *Lamarckiana* to give, as it does, brittle specimens in both cases, but the production of tough mutants and tough hybrids to be a character of our new type *similis*. In order to try this it was, of course, only necessary to cross this new race with *O. rubrinervis*.

I made the cross in 1917, using my typical strain of *O. rubrinervis* as a pollen parent. Next year I had a culture of 180 plants, about one-half of which have flowered, since 111 specimens of the type of *Lamarckiana* had to be removed before the flowering period in order to save space. They constituted three main types: *Lamarckiana* and *lucida*, which were to be expected, and *subrobusta*. No brittle specimen was found. Two mutant individuals occurred, belonging to the types *albida* and *oblonga*. From this we may conclude that the new mutant strain *similis*, which produces *erythrina* instead of *rubrinervis* as mutants, also gives *subrobusta* instead of brittle hybrids in the corresponding crosses.

The seeds of *O. similis* contain the same amount of barren grains as those of *O. Lamarckiana*. In the harvest of the third generation of 1917, I counted 39, 39, 50, and 53 per cent, giving an average of 45 per cent; and in that of the fourth generation in 1921 I counted 60 per cent of empty seeds. This leads to the conception that *similis* has essentially the same constitution as its parent, and consists of *laeta* and *velutina* gametes, but without the factor for brittleness.

We should therefore expect the result of the cross to comply with
the following interpretation: *similis* (=*laeta*+*velutina*)×*rubri-
nervis* (=*deserens*+*velutina*), giving *laeta*×*deserens* (=*lucida*)+
*laeta*×*velutina* (=*Lamarckiana*)+*velutina*×*velutina* (=barren
grains)+*velutina*×*deserens* (=*subrobusta*). Such was exactly the
result. I counted the empty seeds, produced by the cross, on two
individual plants and found 48 and 65, or an average of 56 per cent.
The numerical proportions were found to be, besides the 56 per cent
barren grains, 32 per cent *Lamarckiana*, 9 per cent *subrobusta*, 2
per cent *lucida*, and 1 per cent mutants. These figures differ from
the expectation of four groups of equal size, but the deviations are in
the same sense as in other crosses, the *velutina* (barren grains) giving,
as a rule, too high, but the *lucida* too low figures (8).

In order to compare *O. similis* more closely with *O. Lamarckiana*,
I have studied its crosses with *O. Lamarckiana* mut. *nanella*, and its
mutability after self-fertilization. I failed to discover any differ-
ences. After fertilizing *O. similis* with the dwarfs of my race, I got
in the first generation partly tall plants and partly dwarfs, the num-
ber of the latter being exceptionally high (90–94 per cent). In the
second generation both types gave a uniform progeny. After crossing
the tall hybrids with their dwarfish sisters, I found the same propor-
tion, the offspring consisting of 94 per cent dwarfs and 6 per cent
tall specimens. The types were the same as those of the crosses of
my typical race of *O. Lamarckiana*.

The mutability was studied in the seeds of the second generation,
cultivated as biennial plants in 1913–1914. From five specimens of
*similis* I had after self-fertilization 0.7–1.1 per cent *nanella*, 0.2–0.3
per cent *lata*, 0.3–0.5 per cent *albida*, 0.5–1.0 *oblonga*, a single speci-
men of *semigigas*, but no *rubrinervis*. The seeds of four self-fertilized
plants of 1917 gave, among 936 seedlings, 0.5 per cent *nanella*, 0.9
per cent *lata*, 0.1 per cent *albida*, 1.1 per cent *oblonga*, 1.3 per cent
*scintillans*, and no *rubrinervis*. It is evident that, apart from the
brittleness, the mutability of *O. similis* is the same as that of
*O. Lamarckiana*. We may therefore safely conclude that *O. La-
marckiana* mut. *similis* corresponds in almost all respects with its
parent species, differing only in the factor for brittleness. This latter
is present in *Lamarckiana* but absent in *similis*. For this reason

*O. similis* may be considered to be an atavistic mutant. The ancestors of *O. Lamarckiana* must have acquired the factor for brittleness at some period; their descendants might lose it at any time.

Returning to our formula, the difference between the two types must be looked for in the *velutina* gametes, since the *velutina* × *deserens* give *subrobusta* in the crosses of *O. similis*, whereas the corresponding combination gives *rubrinervis* in the crosses of pure *Lamarckiana*. The three remaining combinations are the same in both cases. In other words, *O. similis* is a mutant which lacks in its *velutina* gametes the factor for brittleness which is so characteristic of *O. Lamarckiana*.

### Oenothera Lamarckiana scindens

This is a race which is externally like a pure *Lamarckiana*, but produces only a very small amount of barren seeds. It originated from a cross made in 1913 between my pure races of *Lamarckiana* and *rubrinervis*. The seeds were sown in 1916, and yielded 32 per cent *Lamarckiana*, 20 per cent *lucida*, and 48 per cent *rubrinervis*. In this respect there was no deviation from the ordinary rule. Of the *Lamarckiana* type, only three specimens were self-fertilized. Two of them produced 66 and 75 per cent of empty seeds, as might be expected. The third, however, had only 7 per cent of such seedlike structures. From this it was evident that a new type had arisen, and a pure strain had since been derived from it. It is to this strain that later the name of *scindens* was given.

According to RENNER (1), the barren seeds of our plants contain small fertilized germs. The failure of their development is due to lethal factors, of which there are two in *O. Lamarckiana*. One of them is linked with the factors for narrow leaves and other characteristics shown in the hybrids of the type *velutina*, and the second one with the broad foliage and other peculiarities of the hybrids of the stature of *laeta*. In *O. Lamarckiana* the amphi-*velutina* and the amphi-*laeta* germs are killed by their respective lethals, and only the combination of the two components produces viable seeds. From these considerations it is evident that the new *scindens* must lack one of the lethals of its parent. Furthermore, the germs of *velutina* are usually produced in too large proportions, but those of

*laeta* in too small numbers, as compared with the figures for ordinary monohybrid splitting (8). Thus the fact that only 7 per cent of barren grains were counted, indicates that the seeds with germs of *velutina* had become viable, while those with *laeta* germs had not suffered any change in their lethal factor. In other words, *O. scindens* is distinguished from *O. Lamarckiana* by the lack of the lethal factor of the *velutina* gametes. It is probable, of course, that this change was accompanied by one or more minor mutations, but these must be left out of consideration in our present discussion. The race must thus split off viable seedlings of *velutina*, besides a small amount of *laeta* germs in the barren grains. The description of its pedigree will prove the exactness of this conclusion. I propose to

TABLE I

PEDIGREE OF OENOTHERA LAMARCKIANA MUT. SCINDENS IN PERCENTAGES

| GENERATION | YEAR | SIZE | BARREN SEEDS | SCINDENS | TARDA | MUTANTS |
|---|---|---|---|---|---|---|
| . . . . . . . . . | 1913 | rubrinervis×Lamarckiana | . . . . . . . | . . . . . . | . . . . . . | . . . . . . |
| 1 . . . . . . . . | 1916 | 1 mutant | . . . . . . . | . . . . . . | . . . . . . | . . . . . . |
| 2 . . . . . . . . | 1917 | 160 Ex. | 7 | 50 | 35 | 8 |
| 3 . . . . . . . . | 1918 | 120 Ex. | 5 | 40 | 48 | 7 |
| 3 . . . . . . . . | 1920 | 121 Ex. | 2 | 43 | 52 | 3 |
| 4 . . . . . . . . | 1921 | 40 | 2 | . . . . . . | . . . . . . | . . . . . . |
| 5 . . . . . . . . | 1924 | 60 | 10 | 51 | 39 | . . . . . . |
| Averages | . . . . . | . . . . . . . . . . . . . . . . . . . . . . . | 5 | 46 | 43 | 6 |

give the name of *O. Lamarckiana* mut. *tarda* to the split off *velutina*, which constitutes a new strain.

I shall first give the pedigree in its most condensed form, indicating the percentages as calculated for the whole harvest, and giving the size of each culture. In 1917 and 1918 almost all of these plants flowered and ripened their fruits, but in repeating the third generation in 1920, only a smaller number were planted out. Every new generation was sown from seeds of a specimen of the type *scindens*, self-fertilized in the previous one. The third generation has been cultivated twice, in 1918 and 1920. The amount of barren grains was determined in glass tubes at about 30° C., containing 100 seeds each.

The mutants in the second generation were *lata, nanella, oblonga, pallescens,* and *lucida*-like, but in 1918 *nanella, oblonga,* and *lucida-*

like, and in 1920 *lata, lucida*-like, and *oblonga*. The *lucida*-like plants
had almost no sterile seeds (0–5 per cent) after self-fertilization;
they indicate the copulation of gametes, mutated into a brittle form.
It is evident that the mutability of *O. scindens* is at least as efficient
as that of the parent species. The loss of the lethal factor for *velutina*
has not changed this quality.

In 1917 I fertilized 28 specimens of the type of *Lamarckiana* with
their own pollen, and counted the barren seeds for each of them.
Their number varied from 0 to 8 in 100 seeds, giving an average of
5 per cent, determined in a total of 2800 seeds. I also counted the
seedlings in the types of *Lamarckiana* and *tarda* for each of the 28
samples separately, and found 33–53 per cent of the first and 47–67
per cent of the second form, with an average of 42 per cent *scindens*
and 58 per cent *tarda*, as determined from about 3000 seedlings. By
the introduction of the barren grains and the mutants into the cal-
culation, these figures become changed into those given in table I.
It is evident, however, that they indicate equal groups of the two
main types. The same conclusion follows from the average of the
harvest of the different generations.

Both *scindens* and *tarda* constituted uniform types, which re-
mained the same during all the generations. No external character-
istics were discovered on which a distinction of *scindens* from *La-
marckiana* could be founded. If we try to express the result of our
cultures in a simple formula, giving the constitution of *O. scindens*,
and call the mutated *velutina* gametes *tarda*, we get the formula:
*O. scindens = laeta + tarda*. In this formula our race is assumed to be
isogamic like its parent. After self-fertilization it must then split
into 50 per cent *laeta × tarda = scindens*, 25 per cent *tarda × tarda*, and
25 per cent *laeta × laeta*, which last constitute the barren grains. The
deviations of our figures from this calculation may be explained by
the fact that *laeta* germs are produced ordinarily in too small num-
bers, whereas the proportion of *velutina* is often far above the expec-
tation (8).

In order to control the exactness of these conclusions, I have
made a series of crosses, partly with other species, and partly with
*O. Lamarckiana* and some of its mutants. The first group produced
hybrids of the types of *laeta* and *velutina*, respectively *densa* and

*laxa*, which were exactly the same as the corresponding hybrids of the parent species, cultivated beside them for comparison. The results are combined into tables II and III. In the case of *O. scindens* × *O. Hookeri*, the seedlings of crosses on four different individuals were separately counted. There were no essential differences, and only the average figures are given. In the cases of *O. scindens* × *O. biennis Chicago*, some of the *laeta* and some of the *velutina* were self-fertilized

TABLE II

CROSSES OF O. SCINDENS WITH OTHER SPECIES

| O. SCINDENS× | CROSS | PERCENTAGE OF | | SIZE OF CULTURE |
|---|---|---|---|---|
| | | laeta | velutina | |
| O. biennis Chicago....... | 1920 | 13 | 87 | 60 Ex. |
| O. Cockerelli........... | 1920 | 33 | 67 | 60 Ex. |
| O. Hookeri............. | 1917 | 38 | 62 | 237 Ex. |
| Average........... | ....... | 28 | 72 | ......... |

TABLE III

CROSSES OF OTHER SPECIES WITH O. SCINDENS

| ×O. SCINDENS | YEAR | PERCENTAGE OF | |
|---|---|---|---|
| | | laeta | velutina |
| O. biennis............... | 1917 | 49 | 51 |
| O. syrticola............. | 1917 | 45 | 55 |
| O. biennis Chicago....... | 1920 | 58 | 42 |
| O. Cockerelli........... | 1917 | 58 | 42 |
| O. Cockerelli........... | 1920 | 43 | 57 |
| O. Hookeri............. | 1917 | 20 | 80 |
| O. Hookeri............. | 1920 | 27 | 73 |
| Average............. | ......... | 43 | 57 |

in 1921; their seeds contained 8 per cent and 2 per cent of sterile grains respectively.

In all these experiments the culture of the hybrids embraced 59–60 seedlings. Those of *O. biennis Chicago* × *scindens* had the types of *densa* and *laxa*. Comparative cultures of the corresponding hybrids of *O. Lamarckiana* were used for the identification. I self-fertilized some specimens in the cultures of 1920, and found only a small amount of barren grains for both types in each of them. From these figures it follows that *O. scindens* has about an equal amount of

gametes of the types of *laeta* and *velutina* among its pollen, but the reciprocal crosses show a deviation from the expectation corresponding to the preferential fertilization in the *velutina* gametes (8).

We may now consider the crosses with *O. Lamarckiana* and some of its mutants. In the first place, I have chosen *O. blandina*, which is a homogeneous race, and three other mutants, in which the active pollen is also homogeneous. I shall first give the numerical results, and afterwards compare them with the expectation as derived from our formula.

The crosses were made in 1920 and 1921, and 60 specimens of each of the cultures have flowered. The first two cases are explained by the formula (*laeta*+*tarda*)×*blandina*, giving *laeta*×*blandina* or

TABLE IV

HOMOGENEOUS CROSSES OF OENOTHERA SCINDENS

|  | PERCENTAGE OF | | BARREN GRAINS |
|---|---|---|---|
| CROSSES | Lamarck-iana-like | blandina and tarda | |
| blandina×scindens....... | 35 | 65 (blandina) | ........ |
| scindens×blandina....... | 12 | 88 (blandina) | ........ |
| scindens×candicans...... | 13 | 87 (tarda) | 2 |
| scindens×secunda........ | 100 | 0 | 11 |
| scindens×elongata....... | 100 | 0 | 8 |

*laeta rediviva*, which is almost like *Lamarckiana*, and *tarda*×*blandina*, which must be expected to be like *velutina*×*blandina*, having the stature of the latter form, and showing an excess of individuals, according to the general rule for both *blandina* and *velutina*.

The cross *scindens*×*candicans* was made in order to prove directly the absence of one of the lethal factors in the *tarda* gametes. *O. candicans*, the formula for which is *O.* (*candicans*+*velutina*)× *velutina* (7), has only pollen of the type of *velutina*, containing the normal lethal of these gametes. If *tarda* possessed the same lethal their combination would yield barren grains, but no viable plants of either type. Barren grains were almost absent, and 87 per cent of the type of *tarda* were produced. This percentage shows the same deviation from expectation as in the two previous crosses, but the main result is that *tarda*×*velutina* gives viable plants, proving that

the lethal of the latter is absent in the former. The other type of the hybrids was *laeta*×*velutina*, giving ordinary *Lamarckiana*.

Secunda and *elongata* are derivatives from *O. simplex* (6). The viable part of their pollen consists of *laeta* gametes only. Combined with *O. scindens*, it must produce *laeta*×*laeta* or barren grains and *tarda*×*laeta* or *scindens*. The barren grains were to be expected in the same percentage as in *O. simplex* itself after self-fertilization, and such was exactly the case. The cultures on the beds were uniformly *scindens*, which, as has been stated, cannot be distinguished in the garden from *O. Lamarckiana*.

A most interesting cross is that with *O. deserens* (4), which is a uniform race, with only one kind of gametes. These contain the factor for brittleness, but have no lethal. Combined with *velutina*

TABLE V

CROSSES OF O. SCINDENS WITH O. DESERENS

| CROSSES | PERCENTAGE OF | |
|---------|--------|---------|
|         | lucida | brittle |
| O. scindens×deserens........... | 15 | 85 |
| O. deserens×scindens.......... | 45 | 55 |

they produce *O. rubrinervis*, from which the strain has originally been split off. The combination with *scindens* must therefore be expected to yield *laeta*×*deserens*, a common hybrid which I have more than once described under the name of *lucida* (4), and *tarda*×*deserens*. This latter combination has the factor for brittleness, which is recessive, on both sides, and must therefore produce brittle plants, which must be expected to comply in their main feature with the type of *O. velutina*×*deserens*=*O. rubrinervis*. Both the expected types of hybrids occurred, as was evident from a comparison with the corresponding hybrids of *O. Lamarckiana*×*O. rubrinervis*.

The crosses were made in 1920, and from each of them I had in the following summer a lot of 60 flowering hybrids. The brittle specimens are in excess, as was to be expected.

With *O. Lamarckiana*, *O. nanella*, and *O. lata*, the results, of course, are of a more complicated nature. I made the combinations shown in table VI in the summer of 1917.

The crosses with *O. nanella* were made with pollen of two differ-

ent strains. The hybrids were counted in the period of well de-
veloped rosettes, in which the distinguishing features were bright
and clear. The barren grains are given in percentages of the whole
harvest. The first two crosses must be expected to give the same
results, since both parents are isogamous. The expectation would
be for equal groups of *laeta* × *laeta* = barren grains, *laeta* × *velutina* =
*Lamarckiana*, *tarda* × *laeta* = *scindens*, and *tarda* × *velutina* = *tarda*.
The types of *Lamarckiana* and *scindens* are exactly the same, and
cannot be separated on the beds; the *tarda* appear in too large num-
bers, as usual.

In the crosses between *Lamarckiana* and *nanella*, a certain
number of dwarfs are usually produced in the first generation, and

TABLE VI

CROSSES OF O. SCINDENS WITH O. LAMARCKIANA, O. NANELLA, AND O. LATA

| CROSSES | PERCENTAGES OF | | TARDA | BARREN GRAINS | SIZE OF CULTURE |
| | Lamarck-iana | nanella, lata | | | |
| --- | --- | --- | --- | --- | --- |
| O. scindens×Lamarckiana.. | 38 | 0 | 62 | 1–11 | 100 Ex. |
| O. Lamarckiana×scindens.. | 50 | 0 | 50 | 4–7 | 100 Ex. |
| O. scindens×nanella....... | 11 | 13 (nanella) | 76 | 8 | 71 Ex. |
| O. scindens×nanella....... | 22 | 20 (nanella) | 58 | 2 | 71 Ex. |
| O. lata×scindens.......... | 43 | 35 (lata) | 22 | 14, 17, 34 | 106 Ex. |

therefore might be expected here also. If we combine them with the
figures for *Lamarckiana*, the results are essentially the same as in
the two previous crosses.

The cross with *lata* may be compared with the crosses of this
mutant with other species, which yield three types of hybrids (3):
*laeta, velutina,* and *lata* in almost equal numbers (6). From this the
expectation for our cross would be: (*laeta* + *velutina* + *lata*) ×
(*laeta* + *tarda*) = *laeta* × *laeta* (empty seeds), *laeta* × *tarda* (= *scindens*),
*velutina* × *laeta* (or *Lamarckiana*), *velutina* × *tarda* (= *tarda*), *lata* ×
*laeta,* and *lata* × *tarda*. The last two must reproduce specimens of
the type of *lata*. If we calculate these figures on the whole harvest,
we get in percentages:

Counted:  21 barren grains; 36 *Lamarckiana* + *scindens;* 16 *tarda;* 27 *lata*

Expected: 17 barren grains; 33 *Lamarckiana* + *scindens;* 17 *tarda;* 33 *lata*

The conformity of the two groups of figures is quite evident.

Considering the results of all our crosses, we find that they give proof of the isogamic condition of our new race *scindens*, and comply with its formula *laeta+tarda*. In this the *laeta* gametes do not show any difference from those of *O. Lamarckiana*, but the *tarda* gametes differ from *velutina* in the absence of the lethal factor.

### Splitting in O. Lamarckiana tarda

About one-third of the seedlings of *O. scindens*, reported in table I for 1917, had the low stature and brittle stems characteristic of *O. tarda*. From one of them I have derived a self-fertilized strain, which I cultivated during three further generations and used for some crosses, in order to study its constitution. In these cultures the *tarda* have split off a new form of the same stature and brittle-

TABLE VII

PEDIGREE OF O. LAMARCKIANA SCINDENS MUT. TARDA

| GENERATION | YEAR | SIZE OF CULTURE | SEED-BEARERS | PERCENTAGE OF PALLIDA |
|---|---|---|---|---|
| 1............ | 1917 | Mutants | 2 | .......... |
| 2............ | 1922 | 115 | 2 | 23 |
| 3............ | 1923 | 60 | 10 | 42 |
| 4............ | 1924 | 600 | .......... | 27 |

ness, but lacking the reddish tinge on the younger parts, and especially on the flower buds, and with broader foliage. I have called this form *pallida*. Table VII records the results of the repeated self-fertilizations in the race of *tarda*.

The percentages are averages from the separate countings of the progeny of the seed-bearers of the previous year. In the third generation I fertilized ten specimens, in order to know whether some of them might have a uniform offspring. This was not the case. The amount of *pallida* differed between 20 and 30 per cent, with an average of 27 per cent.

From table VII we may deduce that the *pallida* is split off after the formula: *tarda* = (*tarda* + *pallida*), which should give, after self-fertilization, 25 per cent of pure *tarda*, 50 per cent of *tarda* × *pallida*, and 25 per cent of pure *pallida*. The first combination must result in barren grains, since no living specimens of it were observed. The

empty seeds amounted to 2–5 per cent in the harvest of 1917, 1–9 per cent for eight of the ten plants of 1923, and 14 and 24 per cent for the two others. On the average the percentage was 8. From this we conclude (8) that the lethal factor was that of the *laeta*, the same as in the parent *scindens* and the grandparent *Lamarckiana*, but which fails in most of the original mutated gametes of *scindens*.

The offspring of the type *tarda* × *pallida* continued the race, while the *pallida* must be uniform and constant, having only recessive characters. In 1922 I fertilized two specimens of it, and had in the two following years the second and third generations, with 60 and 44 flowering plants, all of which were true to type. From this it is clear that no lethal factors could occur in *pallida*. Besides this, some other mutant strains without lethals are known. They are often used in crosses. They are combined in table VIII.

TABLE VIII

HOMOGENEOUS STRAINS DERIVED FROM O. LAMARCKIANA

| A: LAETA | B: VELUTINA |
|---|---|
| 1. *decipiens*, tough | 3. *blandina*, tough |
| 2. *deserens*, brittle | 4. *pallida*, brittle |

The group is thus seen to be completed by our new strain.

In 1922 I made some crosses with *O. tarda* of the second generation, in which I combined it with *O. blandina* and with different species, and had in 1923 a culture of 60 flowering plants for each of them. All of these 480 plants showed the stature and characters of the *velutina* from the corresponding crosses of *Lamarckiana*, which were cultivated on a separate bed near to them, in order to compare them in every phase of their development. No *laeta* plants were found. Only a part of the *velutina*, however, were exactly like their type, since another part had a broader foliage, the leaves reaching about half the breadth of the corresponding *laeta*. Table IX gives the results of the countings.

No brittle plants were found, since this character is recessive. In the first cross the hybrids had the type of *laxa*, as was to be expected. From table IX it is evident that *tarda* is isogamic, the reciprocal crosses giving the same result, and has two kinds of gametes in about equal numbers, corroborating the formula given. The difference between these two kinds of gametes, however, is

much smaller than that between the gametes of either *scindens* or
*Lamarckiana*, almost all of their characters belonging to the category
of *velutina*. This is corroborated by the results of other crosses.
*O. tarda×elongata* gave an almost uniform progeny of the type of
*Lamarckiana*. *Elongata* is a *laeta* type, with uniform pollen, and must
give with the *pallida* the combination *velutina×laeta*, since the
brittleness is recessive. There were 6 per cent of barren seeds and
some mutants.

   *O. tarda×tardescens* produced a dimorphic progeny with some
mutants, which, however, did not flower. Among the 51 flowering
individuals, 39 showed the marks of *tarda* and 12 those of *pallida*.

<div align="center">

TABLE IX

CROSSES OF O. TARDA WITH OTHER SPECIES

</div>

| CROSSES | VELUTINA | BROAD LEAVED |
|---|---|---|
| O. biennis Chicago×tarda.......... | 27 | 33 |
| O. Cockerelli×tarda............... | 26 | 34 |
| O. Hookeri×tarda................. | 33 | 27 |
| O. muricata×tarda................ | 24 | 36 |
| O. blandina×tarda................ | 31 | 29 |
| tarda×O. biennis Chicago.......... | 34 | 26 |
| tarda×O. Hookeri................. | 30 | 30 |
| tarda×O. blandina................ | 27 | 33 |
| Totals....................... | 232 | 248 |
| Percentage................... | 49 | 51 |

*O. tardescens* has only *velutina* gametes in its pollen, and their special
marks were evidently recessive to those of the components of
*O. tarda*.

   *O. tarda×deserens* and its reciprocal also reproduced the two
types of *O. tarda*, among 53 and 60 flowering specimens giving 50–
60 per cent *tarda* and 40–50 per cent *pallida*. *O. deserens* has the
same stature and brittleness as *O. tarda*, but no lethal.

   *O. tarda×O. Lamarckiana* and its reciprocal cross yielded plants
of the types of both parents, in cultures of 54 and 52 flowering
plants giving 40–60 per cent *tarda* and 60–40 per cent of the *La-
marckiana* type. The calculation for this cross is *tarda×laeta*,
*tarda×velutina*, *pallida×laeta*, and *pallida×velutina*. The first com-
bination must give barren grains, the second must give the type of

*tarda*, the third that of *Lamarckiana*, and the fourth combination
evidently results in specimens of the type of *tarda*, since no plants
like *pallida* were found. Thus we see that the different crosses prove
the formula for O. *tarda* = *tarda* × *pallida*, as already given.

### Conclusion

The genetical constitution of the three races may be expressed as
follows: *scindens* = *laeta* + *tarda*; *tarda* = *tarda* + *pallida*; and *pal-
lida* = *pallida*.

The factors involved in the origin of these races are (1) for *laeta*
the same as in O. *Lamarckiana*; (2) for *tarda* the same as in the
*velutina* gametes of O. *Lamarckiana*, with the factor for brittleness,
but with the lethal factor of the *laeta* and without the lethal factor of
the *velutina*; (3) for *pallida* the same as in *tarda*, but without lethal
factors, and with a factor for broader foliage and one for paleness
(small amount of red color). The two latter factors may have been
derived from the *laeta* gametes.

*Pallida* is a homogeneous race, like *deserens*, *blandina*, and
*decipiens*. Of these, *deserens* is also brittle, but in *blandina* and in
*decipiens* this character is absent.

LUNTEREN
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# INHERITANCE OF DEFORMED LEAVES
## IN PHARBITIS NIL

### YOSHITAKA IMAI

#### (WITH ONE FIGURE)

*Pharbitis Nil*, the Japanese morning glory, exhibits several types of variegation. Usually the variegated leaves are more or less deformed by the insertion of white tissues which develop poorly. Another deformed leaf, called "deficient," is a peculiar type of irregularity. The affected leaves are so deformed that they might be mistaken for leaves affected either by disease or an attack of insects. The abnormality, however, is transmitted quite in a Mendelian ratio. The writer will attempt later to discuss the genetic behavior of the variegated and the deficient leaves.

## Two types of variegation

### COMMON VARIEGATION

The recessive nature of the variegated leaf (fig. 1 *A, B*) to the wholly colored condition has been successively proved by several writers, including TAKEZAKI (4), HAGIWARA (1), IMAI (2), and MIYAKE and IMAI (3). In raising an $F_2$ from the self-colored $F_1$, the alternative characters were segregated in the usual ratio of 3:1. Table I gives the total data obtained in the writer's breeding experiments during the past ten years.

The raising of the $F_3$ generation did not give any novel results beyond expectation. The $F_3$ data of two crosses are summarized in table II. This table gives quite the expected results of monohybrid inheritance.

### "GEJIGEJI" VARIEGATION

In several crosses of the variegated and the wholly colored leaves, however, unexpected results were obtained. The parents of these specimens of the crossing having no unusual feature, I naturally expected to obtain therefrom the simplest segregation of 3:1 in the $F_2$ generation. Actually, however, the results were contrary to ex-

pectation, giving the addition of a new type of variegation among
the variegated segregates. As the novelty assumes fine markings of
variegation, it can be differentiated clearly from the common type.
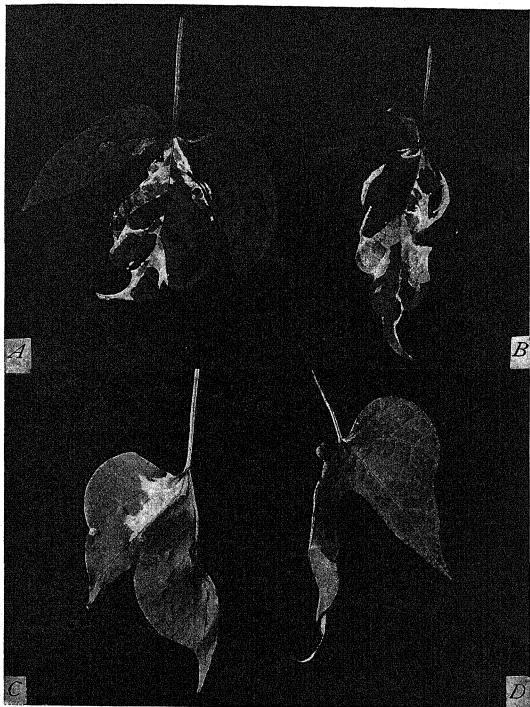


FIG. 1.—A, B, ordinarily variegated leaves, but much deformed; C, D, deficient
leaves; C, variegated, D, self-colored.

TABLE I

| CROSS | SELF-COLORED | VARIEGATED | TOTAL |
|---|---|---|---|
| RA×21-2......... | 62 | 17 | 79 |
| RA×M1......... | 80 | 20 | 100 |
| RA×IH......... | 90 | 16 | 106 |
| β×318........... | 148 | 56 | 204 |
| A×71-2......... | 102 | 37 | 139 |
| β×73........... | 140 | 31 | 171 |
| RA×71-2......... | 219 | 70 | 289 |
| SG×RA......... | 53 | 22 | 75 |
| 319×170......... | 127 | 43 | 170 |
| a×65........... | 81 | 27 | 108 |
| 170×A2......... | 244 | 73 | 317 |
| 170×77......... | 50 | 13 | 63 |
| M2×58-2......... | 99 | 30 | 129 |
| M2×M4......... | 105 | 37 | 142 |
| S3×S5........... | 50 | 13 | 63 |
| 65×505......... | 302 | 84 | 386 |
| WS×AG......... | 37 | 10 | 47 |
| HT×229-1........ | 89 | 20 | 109 |
| N113×HT........ | 44 | 22 | 66 |
| A4×86-2......... | 73 | 20 | 93 |
| 65×A2......... | 124 | 45 | 169 |
| 22-2×A2......... | 28 | 12 | 40 |
| 22-1×A2......... | 124 | 42 | 166 |
| 106×A2......... | 57 | 21 | 78 |
| 22-1×A5......... | 56 | 20 | 76 |
| 11-1×A4......... | 105 | 18 | 123 |
| 318×13-3........ | 85 | 32 | 117 |
| FZ×A2......... | 133 | 29 | 162 |
| NT×A2......... | 196 | 59 | 255 |
| M3×A2......... | 14 | 6 | 20 |
| A1×A2......... | 106 | 28 | 134 |
| 50×A2......... | 279 | 94 | 373 |
| A4×A2......... | 151 | 40 | 191 |
| 324×316......... | 245 | 69 | 314 |
| 319×321B........ | 189 | 61 | 250 |
| 326×A5......... | 490 | 149 | 639 |
| Total.......... | 4605.00 | 1398.00 | 6003 |
| Expected........ | 4502.25 | 1500.75 | 6003 |

TABLE II

| CROSS | CHARACTER OF F₂ | TOTAL OF PEDIGREES | SELF-COLORED | VARIEGATED | TOTAL |
|---|---|---|---|---|---|
| 65×505 | Self-colored............ | 9 | 902 | ......... | 902 |
| | Variegated............. | 35 | 2150 | 656 | 1806 |
| | | 14 | ......... | 780 | 780 |
| 324×316 | Self-colored............ | 20 | 646 | ......... | 646 |
| | Variegated............. | 40 | 900 | 297 | 1197 |
| | | 9 | ......... | 89 | 89 |

This peculiar pattern of variegation reminds one of a Japanese saying "the marks left by the creeping Gejigeji" (a kind of myriapod). We might therefore call this type of variegation "Gejigeji." The observed number of such $F_2$ specimens was composed of three forms, the wholly colored, the commonly variegated, and the "Gejigeji" leaves, which appeared practically in the proportion of 12:3:1, a

TABLE III

| CROSS | SELF-COLORED | ORDINARILY VARIEGATED | "GEJIGEJI" VARIEGATED | TOTAL |
|---|---|---|---|---|
| 314×A2  1............ | 65 | 18 | 4 | 87 |
|             2............ | 27 | 2 | 2 | 31 |
|             3............ | 52 | 22 | 3 | 77 |
|             4............ | 63 | 17 | 10 | 90 |
| RA×314............. | 37 | 2 | 2 | 41 |
| 314×T5.............. | 24 | 6 | 3 | 33 |
| Total.............. | 268.00 | 67.00 | 24.00 | 359 |
| Expected.......... | 269.25 | 67.31 | 22.44 | 359 |

$x^2 = 0.11$; P = practically 1.

TABLE IV

$F_2$ DATA OF CROSS 314×A2

| CHARACTER OF $F_2$ | PEDIGREE NUMBER | SELF-COLORED | ORDINARILY VARIEGATED | "GEJIGEJI" VARIEGATED | TOTAL |
|---|---|---|---|---|---|
| Self-colored............. | A | 18 | 5 | .......... | 23 |
|  | B | 8 | 3 | 1 | 12 |
|  | C | 30 | 7 | 1 | 38 |
| Ordinarily variegated.... | D | .......... | 8 | .......... | 8 |
|  | E | .......... | 15 | 4 | 19 |
|  | F | .......... | 20 | 8 | 28 |
| "Gejigeji" variegated.... | G | .......... | .......... | 5 | 5 |
|  | H | ...:...... | .......... | 6 | 6 |

modified dihybrid ratio (table III). To explain this result factorially the following two allelomorphic pairs may be assumed: (1) $V$, $v$.—$v$ is a factor for the variegation, while its large letter is responsible for the wholly colored condition; (2) $V_g$, $v_g$.—This allelomorphic pair is connected with the "Gejigeji" marking; the $v_g$ factor produces its effect on the $vv$-carrying plants, while on the wholly colored leaves it has no visible effects.

On the assumption that these factors are present, the genetic composition of the $F_1$ plants may be considered to be $VvV_gv_g$. Then we should expect the following combinations of the factors in the $F_2$:

$$(VV_g + Vv_g + vV_g + vv_g)^2 =$$
$$\underbrace{1\,VVV_gV_g + 2\,VvV_gV_g + 2\,VVV_gv_g + 4\,VvV_gv_g}_{\text{9 self-colored}} + \underbrace{1\,vvV_gV_g + 2\,vvV_gv_g}_{\substack{\text{3 commonly} \\ \text{variegated}}} + \underbrace{1\,vvv_gv_g}_{\text{1 “Gejigeji”}}$$

The segregating ratio of the actual numbers corresponds rather closely to the theoretical expectation. The $F_3$ specimens were raised in considerable numbers, but the majority of them were damaged by wandering dogs, which destroyed the seedlings by digging in the field and disturbing the labels. Only a part of them, therefore, which may assist the formulation of the above hypothesis, are given in table IV.

The $F_3$ data thus corresponded quite closely with the expectation. The Gejigeji variegation, due to the combined representation of two recessive factors, was thus fixed at its first appearance. In the $F_2$ segregation of the hybrids of the variegated and the wholly colored leaves, there was commonly the simplest ratio, as was stated in the former section, while in a few cases there appeared the Gejigeji form of variegation in addition to the two parental types. Why should we obtain such different results in apparently the same sort of matings? In considering this problem, it must be remembered that the exceptional crosses were performed invariably by utilizing 314, a pure green leafed specimen, as one of the parents. It was assumed that the factor $v_g$ has no influence on the wholly colored leaf, and its presence cannot be detected by phenotypically examining the self-colored, $VV$-carrying leaves. The pedigree 314 bears the wholly colored leaf, but genetically it carries the $v_g$ factor in the full condition; namely, the genetic composition is $VVv_gv_g$. On crossing this strain with the common variegated one ($vvV_gV_g$), we ought to obtain the self-colored leaves carrying the $VvV_gv_g$ factors as the $F_1$ hybrid, and they actually do result in an $F_2$ consisting of three forms in the $12:3:1$ ratio.

### Deficient leaf

#### APPEARANCE

From a packet of seeds furnished by a seedsman several plants were raised having cordate leaves. On self-fertilization they produced families which bred true to the cordate leaf. Examining the seedlings in the bed, the writer was struck with the unexpected occurrence of a few deformed cotyledons, each forming a certain peculiar shape with fine white splashes on the margin of the affected parts. Encouraged by this discovery, I transplanted all of the seedlings of the abnormal family, and watched their behavior throughout the season. This deformity occurred more frequently on the leaves, but in general its appearance was confined to only a part of the leaves of each individual. On account of the deformed condition of the leaves, this abnormality is called "deficient."[1] This deficient leaf suggests a disease appearance (fig. 1 C, D). The types of the deficient leaf are not confined to any particular shape, but it is possible for any part to be missing. As the deficiency does not appear in all leaves on one individual, however, observation must be made of every leaf throughout the plant growth, from the cotyledons to the upper leaves. The result of such an aberrant family is shown in table V. The deficient leaves thus appeared in only 10.6 per cent, where 25 per cent would be expected in the Mendelian inheritance. The deviation exceeds three times the standard error; so some statement would seem to be needed to account for the source of such an abnormal segregation.

#### EXPERIMENTAL DATA

One of the deficient leaves thus obtained, no. E225, was crossed with a normal leaf (M10). The $F_1$ plants of this reciprocal mating were quite normal, showing no deformed feature. In the following generation there occurred again some deficient leaves. The actual segregation is given in table VI.

The proportion of the deficient leaves is 12.3 per cent, the value being not more than half of that expected. The deviation again exceeds three times the standard error. Such repeated deviations

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[1] The deficient leaf is not a novelty in the Japanese morning glory; this type has figured at times in literature, even in our classical books such as *Asagao Fu* (1830).

cannot be attributed to the result of mere chance sampling. For the sake of convenience of statement, the discussion on this subject will be postponed to later pages. Here let us consider the $F_3$ data. The raising of this generation was made from 62 $F_2$ plants, out of which 56 were normal and the remaining 6 were deficient. The results of these families are given in table VII. When the families including less than 10 individuals are omitted from calculation, the homozygotic and the heterozygotic normals are 13 and 20 respectively, where we should theoretically expect 11 and 22; while 4 normals (nos. 19, 25, 26, and 40), which were previously excluded from this calculation, gave entirely unexpected results. They were apparently quite

TABLE V

|  | NORMAL | DEFICIENT | TOTAL |
|---|---|---|---|
| Observed......... | 93 (89.4 per cent) | 11 (10.6 per cent) | 104 |
| Expected......... | 78 | 26 | 104 |

D = ±15.00; SE = ±4.42.

TABLE VI

|  | NORMAL | DEFICIENT | TOTAL |
|---|---|---|---|
| Observed......... | 138 (87.7 per cent) | 18 (12.3 per cent) | 146 |
| Expected......... | 109.5 | 36.5 | 146 |

D = ±18.5; SE = ±5.23.

normal, yet the greater part of their offspring were composed of deficient leaves, the proportion of the abnormal individuals being 87.6 per cent. Such aberrant results are quite similar to those observed in the progeny of the deficient leaves. Six deficient leaves were taken for raising the $F_3$ generation, and the resultant offspring contained a few normals among the deficient leaves. With reference to each deficient family, some of them bred true to the abnormality, while the others produced some normal rogues. On the average, the abnormal plants appeared in 86.15 per cent of the cases, the other 13.85 per cent being normal.

## OCCURRENCE OF FALSE NORMALS

The abnormal features in the experimental data will be arranged according to four points: (1) the extracted deficient leaves of the

## TABLE VII

F₃ DATA OF CROSS M10×E225

| Character of F₂ | Total of Pedigrees | Normal | Deficient | Total |
|---|---|---|---|---|
|  | 22 | 511 | ............ | 511 |
| Normal | 1......... | 15 | 4 | 19 |
|  | 2......... | 76 | 14 | 90 |
|  | 3......... | 43 | 5 | 48 |
|  | 4......... | 30 | 5 | 35 |
|  | 5......... | 16 | 10 | 26 |
|  | 6......... | 31 | 11 | 42 |
|  | 11......... | 2 | 1 | 3 |
|  | 17......... | 37 | 11 | 48 |
|  | 18......... | 44 | 7 | 51 |
|  | 20......... | 5 | 1 | 6 |
|  | 21......... | 4 | 1 | 5 |
|  | 22......... | 9 | 3 | 12 |
|  | 23......... | 10 | 4 | 14 |
|  | 27......... | 15 | 10 | 25 |
|  | 29......... | 4 | 2 | 6 |
|  | 31......... | 18 | 6 | 24 |
|  | 32......... | 28 | 6 | 34 |
|  | 38......... | 17 | 6 | 23 |
|  | 41......... | 8 | 1 | 9 |
|  | 42......... | 26 | 5 | 31 |
|  | 45......... | 9 | 2 | 11 |
|  | 46......... | 33 | 5 | 38 |
|  | 47......... | 52 | 7 | 59 |
|  | 51......... | 18 | 5 | 23 |
|  | 52......... | 33 | 6 | 39 |
|  | 53......... | 22 | 4 | 26 |
|  | 54......... | 3 | 1 | 4 |
|  | 55......... | 43 | 3 | 46 |
|  | 57......... | 20 | 2 | 22 |
|  | 60......... | 3 | 1 | 4 |
|  | Total...... | 674.00 | 149.00 | 823 |
|  | Expected... | 617.75 | 205.25 | 823 |
| False normal | 19......... | 7 | 13 | 20 |
|  | 25......... | 4 | 28 | 32 |
|  | 26......... | 3 | 48 | 51 |
|  | 40......... | 2 | 24 | 26 |
|  | Total...... | 16 | 113 | 129 |
|  | Expected... | 0 | 129 | 129 |
| Deficient | 10......... | 0 | 15 | 15 |
|  | 30......... | 2 | 6 | 8 |
|  | 34......... | 7 | 36 | 43 |
|  | 39......... | 0 | 1 | 1 |
|  | 50......... | 0 | 2 | 2 |
|  | 59......... | 9 | 52 | 61 |
|  | Total...... | 18 | 112 | 130 |
|  | Expected... | 0 | 130 | 130 |

segregating families were fewer than expected; (2) a few plants, which were noted as normal, gave progenies which included deficient leaves for the large part; (3) the deficient leaves sometimes did not breed true to the abnormality, producing a few individuals which appeared quite normal; (4) the results of (2) and (3) are fundamentally the same, although the phenotype of the parental plants was not common, the one being normal, while the other was deficient.

The following statements attempt to explain these unusual results. As already stated, the deficient feature is not displayed on all leaves of the individual plant; its occurrence is confined to a few leaves only. In such an abnormal state we might expect the occurrence of the exceptional plants which failed to manifest the deficient feature on the leaves throughout their plant growth. As the Japanese morning glory is an annual, dying in the autumn, its life duration is specially limited. If the species were a perennial and made practically unlimited growth, such a false normal might not be obtained. As the so-called false normals are the result of the false representation of the deficient factors throughout their development, they should produce offspring with the same characteristics as those of the deficient plants, frequently producing some false normals among the deficient descendants. In the segregating generation of the normal parents we should also expect the occurrence of false normals. This fluctuating representation should lower the value of the percentage of the deficient leaves in the segregating families.

### FAILING FREQUENCY

To estimate the failing frequency of the deficient representation, all available results are collected in table VIII, where the data of the progeny of the false normals are added on the assumption that they are genetically the same. Thus the estimate shows that the failing frequency on an average is 13.13 per cent.

Next we shall attempt to find the failing value from the data of the segregating normal families. When the value is represented by $x$, the extracted deficient leaves must be $1-x$, and consequently the normal sisters are $3+x$. From this relation we establish the following formula:

$$\frac{\text{number of normal leaves}}{\text{number of deficient leaves}} = \frac{3+x}{1-x}.$$

If we apply the $F_3$ result to this formula, the value of $x$ is 0.4324, or about 43 per cent. Comparing this value with that obtained with the deficient families, the former is more than three times the latter, the difference being too great to be accounted for by mere chance sampling. Then how can we explain it? For the sake of brevity we shall call the failing proportion calculated from the data in the deficient families the "direct value," and that estimated from the results of the segregating progeny of the normals the "indirect value." This designation shows that the segregating aspect of the deficient leaves is different one from the other. In the calculation of the direct value we are dealing with the pure families for the deficient leaf, and as the operation on these was made immediately, the value may not need any qualification. The indirect value, how-

TABLE VIII

|  | NORMAL | DEFICIENT | TOTAL | PERCENTAGE OF NORMAL |
|---|---|---|---|---|
| Observed { from "false normal"...... | 16 | 113 | 129 | 12.40 |
| { from "deficient"......... | 18 | 112 | 130 | 13.85 |
| Total..................... | 34 | 225 | 259 | 13.13 |

ever, depends upon the difference of the theoretical and the observed numbers; so it might include the deviation caused by unequal mortality, or any other such cause as might be present. Strictly speaking, the so-called indirect value of failing frequency, then, might be of absolute frequency plus $a$. As to the reason why such a difference between the direct and the indirect values cannot be attributed to mere chance of deviation, the following statements may be added. The indirect values of the $F_2$ data and the segregating family of unknown origin were 70 and 57.69 per cent respectively. Comparing these frequencies with 43 per cent of the $F_3$ data, the respective difference is not small, and might be accounted for partly by the chance deviation due to the small number of samplings. We cannot neglect the growing condition of the plants in the different years, however, for the influence of the deficient feature may depend largely upon the plant growth. So far as the writer's data showed, the indirect value was always higher than the direct by a consider-

able percentage; this fact may be related to the actual occurrence of unequal mortality between the normal and the deficient leaves, the latter being more damaged than the former in the course of their growth. The problem as to the time when such unequal mortality was introduced (after germination or at gametic or embryonic development), and the problem as to cause (weakness in the struggle or lethal fate) must be left undecided here. If the difference between the direct and the indirect values can be regarded as the result of the influence of unequal mortality, it may represent the actual frequency of the mortality of the deficient leaves. Such a difference amounted to 30.11 per cent in the $F_3$ data, showing that about 30 per cent[2] of unequal mortality occurred in the deficient members of that generation. The reason why only the $F_3$ data are cited for the comparison is that only these were grown in the same year and produced under about the same treatment as was given to the families from which the direct value was calculated.

## Summary

Under the heading of "Deformed leaves" the writer dealt with the genetic behavior of the variegated and the deficient leaves. Summing up, the results are:

1. The variegation is transmitted as a simple recessive to the wholly colored condition.

2. In some crosses of the variegated and the wholly colored leaves there appeared unexpectedly a new type of variegation, called "Gejigeji," a faint variegated pattern.

3. The Gejigeji type is caused by a recessive factor, its dominant allelomorph being a normal variegation on the common basis of the double $v$.

4. The representation of the Gejigeji pattern is limited to the variegated individuals, the effect not being apparent in the self-colored leaves.

5. The ratio in the dihybrid segregation is a 12:3:1 with reference to the wholly colored, the commonly variegated, and the Gejigeji leaves.

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[2] From the fact that the homozygotic and the heterozygotic normals were obtained in the usual 1:2 ratio, this value might not be related to any gametic cause.

6. The effect of the deficient leaf factor appears in both cotyledons and leaves.

7. The deficient leaf looks just like a symptom of some disease, yet it is caused by a genetic factor. The abnormality behaves as a simple recessive to the normal.

8. The representation of the deficient feature is not manifested on all leaves of the plant, only a few leaves in one individual being affected.

9. For this reason there may sometimes be found deficient leaves which are quite normal, failing to show the proper characteristic throughout their plant growth.

10. The false normals may either breed true or produce again a few normals among the deficient offspring.

11. The failing proportion of the deficient leaves is about 13 per cent in the direct calculation, while it attains about 40–70 per cent in the indirect process.

12. The difference of two values may perhaps be mainly due to the unequal mortality of the deficient leaves. If this is the case, the so-called indirect value is of absolute frequency plus $a$.

13. We expect the value to fluctuate depending on the condition of the plant growth.

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# CONTINUOUS RESPIRATION STUDIES OF DORMANT SEEDS OF XANTHIUM

JUNJI OTA

(WITH FOUR FIGURES)

## Introduction

It has been known for some years, from the work of CROCKER (3) and SHULL (6), that the delayed germination of the upper seeds of *Xanthium* is due to certain seed coat and embryo characters connected with the supply and the need of oxygen. The low permeability of the upper seed coat to oxygen, and the high oxygen demand of the upper embryo for germination, act in conjunction in the delayed germination of these seeds. These coat and embryo characters make it possible to keep the upper seeds in germinators for months without germination taking place, unless the seed coats are broken, or the partial pressure of oxygen is increased, or some other source of oxygen supplied, as $H_2O_2$, or the temperature increased sufficiently to overcome the delay due to low oxygen supply.

That there may be other factors in the delayed germination is indicated by certain results reported by SHULL and DAVIS (8), who found that seeds which have been in the germinators for some weeks or months do not germinate as readily as fresh seeds when the coats are removed. The seeds show a tendency to dormancy of the embryos after having been in the germinator for some time without germinating. These writers followed the catalase activity of upper and lower seeds of *Xanthium* during germination and dormancy, and found that long delay of germination while in the germinators leads first to an increased catalase activity of the upper seeds, followed by a decline, which at the end of several months leaves the seeds with no more catalase activity than air dry seeds. The catalase differences between upper and lower seeds were found in harmony with the other physiological differences which cooperate to bring about delayed germination of upper seeds with intact coats. SHULL

and Davis suggested that the respiration rate probably followed the same course as the catalase activity, a rise soon after placing in the germinator, followed by a decline. This suggestion was no doubt based upon a preliminary study of the respiration of upper seeds by Shull (7), and the fact that catalase activity very frequently parallels respiratory rates, as shown by Appleman (1) and others. No study of the respiration of these dormant uppers over long periods in the germinator had been made, however, and indeed the respiration of dormant seeds in general has received but scant attention. Miss Sherman (5) studied the respiration of *Amaranthus retroflexus* seeds over a period of 176 days after harvesting, but she made no continuous studies of the respiration during dormancy, while in germinative conditions. The development of continuous reading respirometers has made it possible to follow the respiratory changes hour by hour, day by day, for long periods. A new design of this type of respirometer is described in this paper.

The work here reported was undertaken to determine the respiration behavior of the upper seeds of *Xanthium* during long periods in the germinators, and to correlate changes in the weight of the seeds during these periods with the $CO_2$ losses in respiration.

## Methods

In order to standardize the conditions of experimentation, the following procedure was adopted. The seeds were kept under constant temperature and moisture conditions at atmospheric pressure, in a Freas thermostat. The seeds were protected from yeasts or other microorganisms by careful handling, but otherwise unsterilized, as some sterilizing agents, like 10 per cent Javelle water, seemed to modify the behavior. At regular intervals the respiration rate was determined, and also weight changes for seeds kept under the same conditions.

The excellent respirometers which have been designed by other workers are not convenient for continuous readings, so an apparatus has been devised for this type of investigation (fig. 1). This consists of three parts, a respirometer chamber, an air tank, and a water manometer. The bulb (*A*) is a respiratory chamber, which is made from a small lamp chimney. This is connected with a storage

bottle (*B*) for sodium hydroxide solution by means of a large rubber tube. This bottle is made from a gooch filter tube, and connected with a short rubber tube to an outlet, which is drawn to a
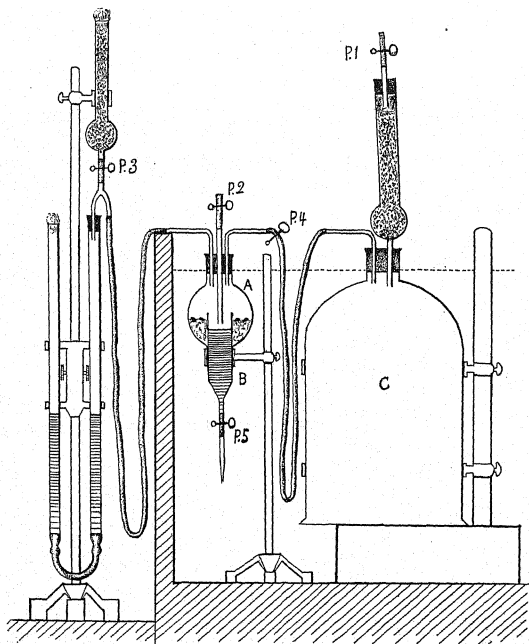


FIG. 1.—Respirometer apparatus.

small opening. The respiratory chamber is also connected by means of tubes to the air tank (*C*) and the leveling water manometer, made of two burettes connected by tubing. The air chamber is made from a large belljar, protected by a soda lime tube, and the burette

manometer was also protected in the same way, as shown in the figure.

The details of the procedure are as follows. The apparatus is first sterilized with formalin solution, and washed with sterilized water. Then the air tank is placed in the water bath, while the pinch clamp ($P_1$) is opened, to drive out the air by water pressure. The tank full of water is now lifted until the lower edge of the belljar is a short distance beneath the surface of the bath. By opening the pinch clamp very slightly, a slow current of $CO_2$-free air is brought into the belljar until it is partially or nearly filled, after which the tank is fastened down securely in the water bath.

The seeds are introduced into the respiratory chamber, and placed on wet absorbent cotton which surrounds the neck of the storage bottle. Opening pinch clamps $P_3$ and $P_2$, a normal solution of NaOH is introduced into bottle $B$ by a pipette discharging through the tube closed by $P_2$. Carbon dioxide-free air from the air tank can be introduced into the respiratory chamber before charging the bottle with NaOH, by opening clamps $P_2$, $P_3$, and $P_4$. The respirometer is then placed in the Freas water bath, the temperature of which was kept at $25°$ C. during the experiments. After any desired interval, the solution of NaOH can be forced from the bottle into a receiving flask through the outlet tube by opening $P_5$ and $P_4$. The water pressure in the air tank, which needs to be only slight, forces the NaOH out of $B$, and simultaneously introduces $CO_2$-free air into the respiratory chamber. Any $CO_2$ which has not yet been absorbed by the solution in $B$ can be swept into the absorbing solution by keeping the pointed end of the delivery tube beneath the surface of the solution in the receiving flask while $CO_2$-free air recharges the respiration chamber.

The water manometer is used to adjust the air pressure of the respiration chamber to atmospheric conditions by opening $P_3$, after which all pinch clamps are closed. Any change of level after the clamps are closed serves as a rough indicator of respiration. It really indicates the amount of oxygen absorbed during the interval. The amount of $CO_2$ evolved in any given interval is determined by the double titration method used by BROWN and ESCOMBE (2) some years ago.

The *Xanthium pennsylvanicum* seeds used were collected at Homewood, Illinois, at the close of the winter season early in 1924. The greatest possible care was used in removing seeds from the burs, so as not to injure the seed coats, and to keep them clean and free from infection. As some sterilizing agents seemed to influence germination behavior, particularly the 10 per cent Javelle water, it was decided not to sterilize the seeds for fear that coat or embryo characters might in some way be affected by the treatment. By careful handling it was relatively easy to avoid any kind of yeast or mold growth, and since the seeds were removed from the burs only immediately before using, bacterial infections were not difficult to avoid.

### Results

The experiments were repeated several times, and always with consistent results. In some cases the seeds were soaked in water in
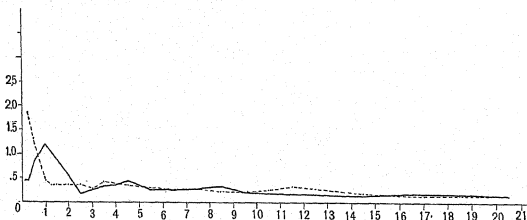


FIG. 2.—Respiration of upper seeds in respirometers: horizontal axis represents time in days, vertical axis represents mg. of $CO_2$, continuous line represents unsoaked seeds, broken line represents soaked seeds.

an icebox over night previous to placing them in the respirometer, while in other cases they were introduced into the respirometer dry, so that soaking occurred on the wet absorbent cotton in the chamber. The experiments were run for three weeks each. While this is a relatively short period, it is clear from the consistent behavior of the seeds that the course of respiration would continue indefinitely along the lines noted during the last two weeks of this period.

The data for the respiration of unsoaked seeds are shown in table I, and for soaked seeds in table II. Both results are shown graphically in fig. 2.

## TABLE I

### RESPIRATION OF UNSOAKED XANTHIUM SEEDS

| TIME IN HOURS | CO₂ GIVEN OFF BY 20 SEEDS (MG.) | CO₂ GIVEN OFF BY 20 SEEDS PER HOUR (MG.) | No. OF GERMINATIONS |
|---|---|---|---|
| 0–4 | 1.7600 | 0.4400 | |
| 4–8 | 1.7600 | 0.4400 | |
| 8–13 | 3.5200 | 0.7040 | |
| 13–24 | 13.2000 | 1.2000 | |
| 24–36 | 8.8000 | 0.7333 | |
| 36–48 | 6.1600 | 0.5133 | |
| 48–60 | 2.2000 | 0.1833 | |
| 60–72 | 2.6400 | 0.2200 | |
| 72–84 | 3.9600 | 0.3300 | |
| 84–96 | 4.4000 | 0.3667 | |
| 96–108 | 4.8400 | 0.4033 | |
| 108–132 | 6.6000 | 0.2750 | |
| 132–156 | 6.6000 | 0.2750 | |
| 156–180 | 6.6160 | 0.2757 | |
| 180–204 | 7.4800 | 0.3117 | 1 |
| 204–228 | 5.0600 | 0.2108 | |
| 228–252 | 4.6200 | 0.1925 | |
| 252–276 | 4.1480 | 0.1728 | |
| 276–300 | 3.6859 | 0.1536 | |
| 300–348 | 6.6000 | 0.1375 | |
| 348–396 | 7.920 | 0.1650 | 1 |
| 396–444 | 7.040 | 0.1467 | |
| 444–492 | 6.600 | 0.1375 | |

## TABLE II

### RESPIRATION OF SOAKED XANTHIUM SEEDS

| TIME IN HOURS | CO₂ GIVEN OFF BY 20 SEEDS (MG.) | CO₂ GIVEN OFF BY 20 SEEDS PER HOUR (MG.) | No. OF GERMINATIONS |
|---|---|---|---|
| 0–6 | 11.0000 | 1.8333 | |
| 6–12 | 4.8400 | 0.8067 | |
| 12–24 | 5.2800 | 0.4400 | |
| 24–30 | 2.2000 | 0.3667 | |
| 30–36 | 2.2000 | 0.3667 | |
| 36–48 | 4.4000 | 0.3667 | |
| 48–60 | 4.2000 | 0.3500 | |
| 60–72 | 3.5200 | 0.2933 | |
| 72–84 | 4.8400 | 0.4033 | |
| 84–108 | 8.8000 | 0.3667 | |
| 108–132 | 7.0400 | 0.2933 | |
| 132–156 | 7.0400 | 0.2933 | |
| 156–180 | 6.6000 | 0.2750 | |
| 180–204 | 5.2800 | 0.2200 | |
| 204–228 | 4.8400 | 0.2017 | |
| 228–252 | 6.1600 | 0.2567 | |
| 252–276 | 7.4800 | 0.3117 | 1 |
| 276–300 | 7.0400 | 0.2933 | |
| 300–348 | 9.2400 | 0.1925 | |
| 348–396 | 7.4800 | 0.1558 | |
| 396–444 | 7.2000 | 0.1500 | |
| 444–492 | 6.6000 | 0.1375 | |

It is seen from the tables and graphs that the respiration rate rises rapidly during the first day or so of germinative conditions, reaching a maximum during this brief period. After this initial

TABLE III

RESPIRATION AFTER REDRYING AND WEIGHT LOSS

| TIME (HOURS) | $CO_2$ GIVEN OFF BY 20 SEEDS (MG.) | $CO_2$ GIVEN OFF BY 20 SEEDS PER HOUR (MG.) | DRY WEIGHT OF 20 SEEDS (MG.) |
|---|---|---|---|
| 0–6............. | 4.4000 | 0.7333 | 946.0 |
| 6–12............. | 7.0400 | 1.1733 | (initial weight) |
| 12–24............. | 8.8000 | 0.7333 | |
| 24–30............. | 4.8400 | 0.8067 | |
| 30–36............. | 3.5200 | 0.5867 | |
| 36–48............. | 3.0800 | 0.2567 | |
| 48–60............. | 3.0800 | 0.2567 | |
| | Total.. 34.7600 | Average. 0.5793 | |
| 60–108............. seeds dried to air dry weight | | | 870.0 |
| 108–114............. | 2.4720 | 0.4620 | |
| 114–120............. | 4.0900 | 0.6817 | |
| 120–132............. | 6.0060 | 0.5005 | |
| 132–138............. | 3.8000 | 0.6333 | |
| 138–144............. | 3.0800 | 0.5133 | |
| 144–156............. | 3.2300 | 0.2692 | |
| 156–168............. | 3.1700 | 0.2642 | |
| | Total.. 25.8480 | Average. 0.4341 | |
| 168–216............. seeds dried to air dry weight | | | 845.0 |
| 216–222............. | 2.8600 | 0.4767 | |
| 222–228............. | 4.4000 | 0.7333 | |
| 228–240............. | 3.9600 | 0.3300 | |
| 240–246............. | 1.9800 | 0.3300 | |
| 246–252............. | 3.5000 | 0.2917 | |
| 252–264............. | 3.9600 | 0.3300 | |
| 264–276............. | 3.0800 | 0.2567 | |
| | Total.. 23.7400 | Average. 0.3957 | |
| 276–324............. seeds dried to air dry weight | | | 822.3 |

increase the respiration rate falls rapidly, with some slight fluctuations during the second and third days, to a low level, after which there is a very slow decrease in rate as long as the experiment is continued.

The graphs also show clearly that the soaked seeds reach their maximum respiration rate much sooner than dry seeds. It only takes about 6 hours for the soaked seeds, but 12–24 hours for the dry seeds to reach the point of most rapid evolution of $CO_2$. If at any time a seed germinated unexpectedly, there was always a corresponding increase in the respiration. Such seeds were removed from the respirometer as soon as detected.

The effect of redrying upon the respiration rate was tested. After the seeds had reached the low level of respiration in the respirometer, they were removed and dried, and then used again for an experiment. It was thought that they might again show an

TABLE IV

RESPIRATION AND WEIGHT LOSS IN XANTHIUM SEEDS

| No. of lot (10 seeds) | Initial dry weight (gm.) | Time in hours | Decrease in weight | Decrease in wt. per gm. |
|---|---|---|---|---|
| 1.............. | 0.3309 | 6 | 0.0108 | 0.0326 |
| 2.............. | 0.3640 | 12 | 0.0168 | 0.0462 |
| 3.............. | 0.4127 | 18 | 0.0197 | 0.0478 |
| 4.............. | 0.5528 | 24 | 0.0368 | 0.0666 |
| 5.............. | 0.4080 | 36 | 0.0320 | 0.0784 |
| 6.............. | 0.3711 | 48 | 0.0321 | 0.0865 |
| 7.............. | 0.4240 | 144 | 0.0340 | 0.0800 |
| 8.............. | 0.3903 | 288 | 0.0370 | 0.0874 |
| 9.............. | 0.3720 | 480 | 0.0386 | 0.1038 |

initial high respiration rate. The results of tests are given in table III and fig. 3, which show that the expected initial rise in respiration can be obtained repeatedly by redrying and subjecting to germinative conditions.

The weight changes accompanying long continued respiration of dormant seeds have been followed in five series of experiments, which were frequently disturbed by the unexpected germination of seeds. The results shown in table IV and fig. 4 are compiled from three series of tests. The initial weight is taken as the air dry weight, but in determining the loss in weight the seeds were oven dried at 103° for 5 hours after drying at room temperature for 24 hours.

The general result shows a relatively rapid decrease in weight during the first several days, but after this the rate decrease is slow,
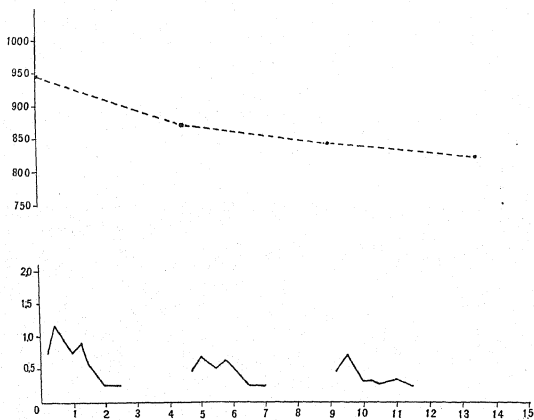
FIG. 3.—Respiration of redried seeds: horizontal axis represents time in days, vertical axis represents weight in mg., continuous line represents respiration rate, broken line represents weight change.
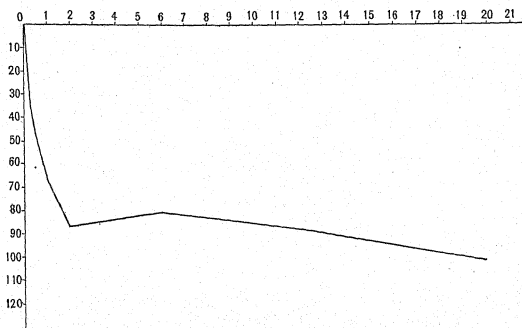


FIG. 4.—Weight changes of upper seeds: horizontal axis represents time in days, vertical line represents decrease of weight in mg. per gram of seeds.

corresponding to the slow respiration rate as determined from $CO_2$ evolution.

<h2 style="text-align:center">Discussion</h2>

As was mentioned in the introduction, a study of respiration of dormant seeds was made by Miss SHERMAN (5), who determined experimentally the respiratory intensity, that is the mg. $CO_2$ eliminated per gram of imbibed seeds per hour for *Amaranthus retroflexus*, *Chenopodium album*, and *Rumex crispus*, as well as for *Crataegus* and certain drupaceous Rosaceae. Her data show that the amount of $CO_2$ evolved per gram of imbibed weight per day at 25° C. is between 1.548 and 4.213 mg. for these seeds. The respiratory intensity varies markedly for different kinds of seeds. She made no studies of *Xanthium* seeds, and in no case were the seeds studied continuously while in germinative conditions. The work here reported, therefore, is not directly comparable with Miss SHERMAN'S work, as it deals with a somewhat different phase of respiratory behavior, the long continued respiration of dormant seeds kept in conditions otherwise suitable for germination.

These experiments show that the dormant upper seeds of *Xanthium* show a high respiration rate during the first few days. If the data of the first 60 hours of table III are taken, the amount of $CO_2$ eliminated per gram of dry seeds per 24 hours at 25° C. is 14.48 mg., but if the data at the twentieth day be taken, the evolution of $CO_2$ is only about one-tenth as great.

Many plant and animal physiologists have found a parallelism between catalase activity and respiratory intensity. Miss SHERMAN, however, found instances in which there was no direct correlation between these two activities. Fluctuations in the two did not occur simultaneously, and might be in opposite directions. More recently, Mrs. RHINE (4) has found that respiratory and catalase activity go in opposite directions in the earliest stages of germination in all the types of seeds tested.

In the experiments of SHULL and DAVIS it was found that the upper seeds of *Xanthium* showed increased catalase activity during the first two days in the germinator, but as the period of exposure lengthened, the activity decreased to less than one-third at 43 days, and at 93 days was no greater than in seeds kept in air dry storage.

When a seed was broken and began to germinate after 42 days, the catalase activity increased suddenly.

Inspection of the respiration curve in fig. 2 shows that the respiration behavior in the respirometer is very similar to the catalase behavior in the germinator. In these later stages, it is certain that the catalase activity and respiratory activity run closely parallel throughout the period of dormancy.

The fact that the respiration rate of soaked seeds reached its maximum in the respirometer earlier than that of unsoaked seeds, is mainly due to the fact that the unsoaked seeds needed time to take in sufficient water for destructive metabolism, while soaked seeds have already imbibed sufficient water for this purpose. It is well known that the dry weight of ordinary seeds decreases rapidly during germination, while respiratory activity increases. In the case of dormant seeds the same relations exist; if respiratory activity is rapid, weight loss is rapid; when the respiration is slow, weight decrease is slow. Calculations based on the data presented show a more rapid weight loss than actually occurs in long time experiments, which can only mean that the respiratory loss and weight loss continue to decline until the daily loss is insignificant. Even if the twentieth day rate of respiration is taken, the evolution of $CO_2$ found would be sufficient to exhaust the carbon of the seed in a couple of months, counting the seed 50 per cent carbon. They have been kept in germinators for 93 days without exhausting the reserve food, however, and in nature must be able to withstand at least several years of slow respiration without complete exhaustion of the food carbon.

## Summary

1. There is a notable increase in the respiration rate of dry seeds during the first day of subjection to favorable germinative conditions in the respirometer.

2. After this initial increase, the respiration rate falls rapidly, with some slight fluctuations during the second and third days, to a low level, after which there is a very slow decrease in rate as long as the experiment is continued.

3. If the seeds are soaked in cold water previous to placing them in the germinator, the respiration rate reaches its maximum earlier

than unsoaked seeds; otherwise the curve of respiratory activity is the same as in unsoaked seeds.

4. If the seeds are taken from the respirometer and dried, and again placed in the respirometer, they always show the increased respiration immediately after being placed in germinative conditions.

5. The weight changes occurring in seeds during long periods of dormant respiration have also been noted and compared with the respiration data. The experiments did not run long enough to give the natural rate of carbon loss from respiration in nature.

6. Respiratory activity and catalase activity in these seeds run parallel throughout the period of dormancy.

The writer desires to express his appreciation and gratitude to Professor CHARLES A. SHULL, under whose direction these experiments were carried on, and to Dr. S. V. EATON for many suggestions during the course of the work.

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# NOTES ON THE "SPRUCE BUDWORM" BIOCOENOSE

## II. STRUCTURAL ABNORMALITIES IN ABIES BALSAMEA

I. W. BAILEY

(WITH PLATES XIII–XV AND THREE FIGURES)

### Introduction

As noted in the first paper of this series,[1] analyses of the growth rings in the stems of spruce and fir balsam have afforded useful criteria in studying the activities of the budworm, *Cacoecia fumiferana*, in the coniferous forests of Eastern Canada. In the case of the fir balsam, the problem of accurately dating the layers formed during specific growing seasons is complicated by the occurrence of dark reddish brown zones, which tend at times to simulate the outer boundaries of true growth layers. It is essential to determine whether these apparent abnormalities are induced by the feeding of the budworm, and to obtain more reliable information concerning their significance in stem analyses.

### Macroscopic investigation

In transverse sections of normal, uninjured stems of fir balsam, the two or three innermost growth layers (those next to the pith) are characterized by having a more or less intensified, dark reddish brown color. The "discoloration" may be diffused throughout the growth layers, confined to broad zones in their outer portions, or distributed sporadically in irregular patterns. It becomes evanescent in the three or four succeeding growth layers, and disappears in subsequently formed tissue.

In sections of fir balsam which have been attacked by the budworm, there are at times two or three sharply defined, narrow, dark reddish brown zones. These discolorations are not restricted to the innermost growth layers, however, but may be present in various portions of the tissue differentiated during the first 8–12 growing seasons. An examination of series of transverse sections, from differ-

ent levels of the cauline axis, reveals the fact that discolorations of this type are confined to the upper extensions of growth layers, formed subsequent to the advent of the budworm. This, of course, suggests that they are induced either directly or indirectly by the feeding of the insects. In most cases the defoliating activities of the budworm extend over a period of from two to four years. Each season the young larvae feed upon the tender, recently formed leaves, and subsequently consume a varying proportion of the older foliage. Thus the injurious effects of defoliation tend to be intensified in the terminal shoots. That the formation of the narrow, zonal discolorations is more or less closely correlated with injuries to the apical shoots, is indicated by a detailed investigation of such specimens as those illustrated in text figs. 1 and 2. The terminal shoot in text fig. 1 was severely injured by the feeding of the budworm, and subsequently was replaced by a lateral shoot which curved upward into a vertical position. Fig. 4 is a transverse section of the stem cut at level *a*. The narrow, innermost layer is jacketed by two broad growth rings of normal, circular outline. The succeeding ring also is cylindrical, but is much reduced in width. The six outer layers are strikingly eccentric, and are characterized by having conspicuous arcs of so-called compression wood or Rotholz. HARTIG[2] and others have shown that this type of eccentricity is closely correlated with the upward bending of lateral shoots. It is evident, therefore, that the abnormally narrow, cylindrical growth layer in fig. 4 must have been formed during the growing season in which the injury to the apical shoot occurred. This layer has a narrow, dark reddish brown zone of discoloration close to its outer margin. The discoloration is visible in sections from a number of successively lower levels. In other words, it has a considerable longitudinal extension below the stub of the terminal shoot. It should be noted, in this connection, that its upper portions are closer to the pith than are its basal extensions. This, of course, is due to the fact that the layer, to which it is confined, jackets an increasing number of previously formed growth rings at successively lower levels.

In the specimen illustrated in text fig. 2, there are evidences of

[2] HARTIG, R., Holzuntersuchungen: Alters und Neues. Berlin: J. Springer. 1901 (pp. 46–99).

three successive injuries to shoots in the apical portions of the stem.
The differentiating terminal and lateral shoots at level $b$ were
severely injured during the first year (1914) of feeding of the bud-
worm. The apical shoot survived this attack, but was so severely
injured during the succeeding growing season that it was replaced
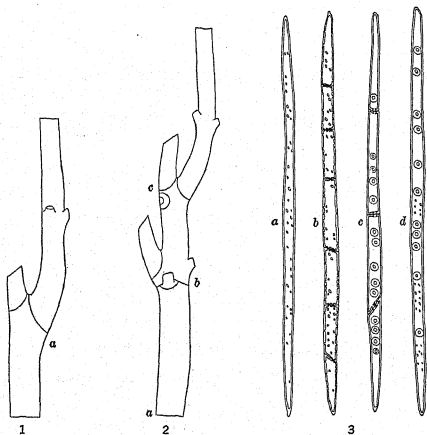


FIGS. 1-3.—Fig. 1, terminal portion of stem of fir balsam seriously injured by
defoliation in 1914; during the 1915 growing season a lateral shoot curved upward to
replace injured terminal shoot; fig. 2, apical portion of stem of fir balsam, showing
successive malformations due to feeding of budworm; fig. 3, types of cells formed by
fusiform initials of cambium: $a$, simple pitted, unsegmented parenchymatous deriva-
tive; $b$, simple pitted, segmented parenchymatous derivative; $c$, septate tracheid;
$d$, normal, unsegmented tracheid.

by one of the laterals, level $c$. Less conspicuous malformations were
produced in the following year. Fig. 3 is a transverse section of this
stem cut at level $a$. There is a narrow zone of discoloration in each
of the 1914–1916 growth layers. As in the preceding specimen,
these discolorations, which have a considerable longitudinal exten-
sion, are confined to growth layers formed during seasons in which
injuries to the apical shoots occurred.

## Microscopic investigations

As shown in figs. 5–7 and figs. 9–12, the dark reddish brown discolorations are due to the presence of cells which contain an amber colored substance (appearing black in the photomicrographs). In the case of the broader, less symmetrical discolorations, that is, those which are not confined to growth layers formed subsequent to the advent of the budworm, these "resin" cells are more or less widely and irregularly scattered (fig. 5). On the contrary, in the case of the discolorations which are correlated with injuries to the apical shoots, they are aggregated in relatively narrow zones (figs. 6, 7, 10, 11, 12), and not infrequently are associated with traumatic "resin" cysts (figs. 6, 7, 10, 12).

Two types of vertically elongated, "resin"[3] bearing cells are known to occur in the secondary wood of the Coniferales, resinous tracheids and resiniferous wood parenchyma. The former are normal tracheids into which resinous material is supposed to have diffused from the adjoining ray parenchyma. The "resin" occurs in the form of more or less massive, biconcave, transverse septa. Such resinous cells are considered characteristic features of the wood of certain representatives of the Cordaitales and araucarian conifers, but may occur at times in *Abies* and *Pinus*, as PENHALLOW[4] and RECORD[5] have shown. Wood parenchyma is present in many of the higher gymnosperms. In certain plants it tends to be confined to the outer surface of the so-called summer wood, whereas in others it is more or less regularly diffused throughout the secondary wood. In the cells of this tissue, the resinous material, which varies greatly in form and color, is presumably *in situ.*

In the case of the discolorations which occur in the inner growth layers of normal, uninjured fir balsams, the resin bearing elements appear at first sight to be tracheids. This is due to the fact that they are of similar size and form, and that their pitting is obscured by

[3] The words resin, resinous, resiniferous, etc., frequently are used in referring to substances of unknown chemical composition. Many of these amber colored substances do not exhibit the typical reactions of true resins.

[4] PENHALLOW, D. P., A manual of the North American gymnosperms. Boston: Ginn & Co. 1907. pp. 53–58.

[5] RECORD, S. J., Significance of resinous tracheids. BOT. GAZ. 66:61–67. 1918.

their amber colored contents. A detailed study of their secondary membranes, however, shows them to be provided with small, simple openings (text fig. 3 and fig. 17) instead of with large circular bordered pits. The occurrence of such cells in the secondary xylem of the gymnosperms, so far as the writer has been able to determine, is unique. It raises the question whether they should be designated as resinous libriform fibers, as resinous substitute fibers, or as structures *sui generis*.

In the narrow zones of discolored tissue which are confined to growth layers formed subsequent to the advent of the budworm, these cells occur in association with normal tracheids (fig. 13), septate tracheids (fig. 14), resiniferous wood parenchyma (fig. 15), or traumatic resin cysts (fig. 16). In general, the amber colored substance is confined to elements having simple pits, and, in most cases at least, does not diffuse into the tracheary cells. In other words, the cambium forms two distinct categories of vertically elongated cells, tracheids and simple pitted elements which tend to remain physiologically active during a longer period and to secrete an amber colored substance. Both types of cells are derived from the same fusiform cambial initials. Furthermore, in both categories of elements, the fusiform daughter cells of the cambium may divide transversely before differentiating into bordered pitted or simple pitted elements (text fig. 3). It is significant, in this connection, that the frequency of such transverse divisions appears to vary directly with the quality or the intensity of the traumatic stimulus. For example, septate tracheids and resin bearing cells in close proximity to "resin" cysts tend to be composed of short segments, whereas the outlying cells are nonseptate or exhibit two or three transverse divisions.

Such facts suggest that the simple pitted cells in the innermost rings of normal, uninjured fir balsams are essentially parenchymatous, and may be designated as nonseptate or unsegmented wood parenchyma. Their resemblance to tracheary elements is due to the fact that both types of cells are derived from fusiform cambial initials, and therefore are of similar form. It should be emphasized in passing that the significance of the shape of the cambial initials has been overlooked in phylogenetic speculations concerning the origin of wood parenchyma. For example, the fact that series of

vertically contiguous parenchymatous cells have a fusiform outline, resembling that of tracheary elements, has been advanced as indicating that wood parenchyma is primitively derived from modified tracheids. Such an assumption, of course, is entirely unjustifiable. Nor is the occurrence of short tracheids in association with parenchymatous elements necessarily indicative of the derivation of wood parenchyma from septate tracheids. There is an evident tendency toward the replacement of tracheary by parenchymatous elements in the secondary xylem of many of the higher gymnosperms, but there is no reliable evidence of an evolutionary metamorphosis of tracheids into wood parenchyma, that is, that tracheids became septate and subsequently lost their bordered pits and reacquired living contents. Even in the case of the dicotyledons, it is a question whether the so-called substitute fibers actually are modified tracheary elements, or are elongated, thick walled, unsegmented wood parenchyma.

As shown in figs. 13–17, the amber colored substance in the parenchymatous elements of fir balsam occurs in various granular, alveolar, and more or less massive forms. It not infrequently appears as an inner jacketing layer with numerous transverse septa of varying width (fig. 13). The more massive, biconcave septa (figs. 13–15) closely resemble those that occur in the resinous tracheids of the Cordaitales and Araucarieae. The tenuous types (figs. 13, 17) give to the elements in which they occur the appearance of being divided into short segments.

### Significance of reddish brown discoloration in stem analyses

Although the narrow, zonal discolorations are of considerable value in distinguishing tissue formed subsequent to the advent of the budworm, in many cases they are serious obstacles in studying the chronology of the successively formed growth layers. This is due to the fact that, when they are formed at the end of the growing season, they tend to obscure the outer margin of the growth ring, and, when they are differentiated earlier, they tend to divide the growth ring into two apparently distinct layers. The difficulties in distinguishing the outer margins of true growth layers, of course, are considerably accentuated in macroscopic analyses, but cannot in all

cases be overcome by a microscopic examination of specific transverse sections.

In fig. 7, the inner growth layer terminates in a zone of narrow tracheids, which is subtended by a conspicuous zone of traumatic tissue. There is an inner zone of diffusely scattered "resin" cells, which are associated with enlarged tracheids. All of this tissue was formed during a single growing season (1911), and there is no uncertainty concerning the interpretation of its principal topographical features. The same thing is true of the inner (1911) growth layer in fig. 6. It terminates in a conspicuous zone of summer wood, which is subtended by a zone of aggregated wood parenchyma. The succeeding tracheary tissue is divided into two portions by a narrow zone of traumatic "resin" cysts and "resin" cells. There is a slight tendency toward a reduction in the width of the tracheids in the vicinity of this zone of discolored tissue, but there is no reliable structural evidence to indicate whether the zone of discoloration was formed during or at the end of the growing season. Similar difficulties are encountered in interpreting the structures in figs. 11 and 12.

It occurred to the writer that it might be possible to overcome such uncertainties by tracing the zones of discoloration downward into the level of the stem at which they disappear. At their lower extremities, the outer zones of traumatic tissue in figs. 6 and 12 grade into conspicuous zones of summer wood. On the contrary, the outer of the two central zones of traumatic "resin" cells in fig. 11 fades away in what appears to be the inner portion of a growth layer, and is not subtended longitudinally by a zone of dense tissue. This suggests that the zonal discolorations in figs. 6 and 12 were formed at the end of the growing season, and therefore coincide with the outer margins of true growth layers; but that one of the putative layers in fig. 11 is a false ring. It should be emphasized, however, that the occurrence of tracheids of reduced radial width in fig. 11 raises the question whether the dense layers which subtend the outer discolorations in figs. 6 and 12 are false zones of summer wood induced by the feeding of the budworm. The question may be answered by accurately dating the growth layers, for example, by the method outlined in the first paper of this series. In

the specimens illustrated in figs. 6 and 12, there is one layer for each
of the 1911, 1912, and 1913 growing seasons. On the contrary, in
fig. 11 the 1917 growth ring is divided into two apparently distinct
layers.

A detailed study of a large number of fir balsams from various
forest areas in western and south central Quebec, and from northern
New Brunswick indicates that broad, conspicuous "false" layers of
summer wood are of rare occurrence in trees attacked by the bud-
worm. When present, they rarely extend around the entire cir-
cumference of the stem, and are of much restricted longitudinal
distribution. Thus, they do not present a serious practical obstacle
in stem analyses. Of greater significance is the fact that cambial
activity may be arrested during one or more growing seasons, par-
ticularly in the median and basal portions of the cauline axis. A
reduction in the number of layers, therefore, in passing from a higher
to a lower level of the stem, may be due to the presence of a false
ring (fig. 11) at the former level, or to the omission of a growth
layer at the latter level. It should be noted in this connection, how-
ever, that if the two 1917 layers in fig. 11 were distinct annual rings,
one of which disappeared at a lower level, the dividing zone of trau-
matic tissue would not terminate in the central portion of a growth
layer. If the inner layer became evanescent at lower levels, its outer
margin would gradually approach that of the preceding ring. Sim-
ilarly, if the outer layer faded away at lower levels, its outer mar-
gin would approach the zone of tissue in which the discoloration
occurs.

It is evident, therefore, that there are two methods of studying
the chronology of the growth layers in stems of fir balsams that have
been attacked by the budworm: (1) by using frost rings as indi-
cators, and (2) by carefully tracing the growth layers and the zones
of discolored traumatic tissue downward into successively lower
levels of the stem. The former method necessitates an extensive
preliminary examination of the distribution of frost injuries in the
vegetation of specific forest areas; the latter involves a careful dis-
section of the cauline axis, particularly of its slender, upper portion.
Either method may be used in the case of trees which have recov-
ered from the attacks of the budworm. In the case of trees which

have been killed by defoliation, or of dying trees in which the date
of formation of the outermost ring is not accurately known, both
methods are essential.

### Structural abnormalities produced by Pissodes dubius

The weakened and dying trees in coniferous forests which have
been defoliated by the budworm are attacked by various insects and
fungi. One of the commonest of these secondary parasites is *Pissodes dubius*. The adult weevils bore into the stems of fir balsam
and feed upon the inner phloem and cambium. In so doing, they
excavate shallow, oval cavities which later may become more or less
completely occluded by wound tissue (fig. 2). If the bark is removed
from the stems of trees which were attacked by the weevils during the
preceding growing season, the surface of the woody cylinder will be
found to be covered with narrow, vertically elongated, dark reddish
brown streaks. These discolorations originate in the vicinity of the
injuries produced by the feeding of *Pissodes*, and extend a consider-
able distance above and below them. As indicated in figs. 1 and 8,
they are due to the presence of traumatic "resin" cysts whose epi-
thelial cells contain an amber colored substance.

The feeding scars and associated discolorations are of such char-
acteristic form and distribution, that they may be utilized in tracing
the former activities of the weevils in different regions. For example,
in the case of trees which have fully recovered from the effects of the
earlier outbreaks of the budworm (1909, 1910, 1911, etc.), the occur-
rence of these abnormalities in the narrower growth layers, formed
subsequent to the advent of the budworm, indicates that the trees
were attacked by *Pissodes* before they recovered from the effects of
defoliation. Similarly, in the case of dead and dying trees, it is pos-
sible to determine the dates of the growing seasons during which the
weevils were actively feeding upon the trees.

### Summary

1. In transverse sections of the stems of fir balsams that have
been attacked by the spruce budworm, there frequently are two or
three narrow, dark reddish brown zones of discolored tissue.

2. These discolorations are due to the presence of parenchy-

matous elements which contain an amber colored substance. They
are confined to the upper extensions of growth layers formed subse-
quent to the advent of the budworm, and are induced by injuries
to the terminal shoots.

3. The abnormalities not infrequently are serious obstacles in
studying the chronology of the successively formed growth layers of
the cauline axis. Two methods of overcoming such difficulties in
stem analyses are briefly outlined.

4. The weakened and dying trees in coniferous forests that have
been defoliated by the budworm are attacked by various insects and
fungi. One of the commonest of these secondary parasites is *Pis-
sodes dubius*. It is shown that the areas of traumatic tissue induced
by the feeding of this weevil are of considerable significance in stem
analyses.

The data presented in this paper were collected during a recon-
noissance for the Entomological Branch of the Canadian Depart-
ment of Agriculture.

BUSSEY INSTITUTION,
FOREST HILLS, MASS.

## DESCRIPTION OF PLATES XIII–XV

FIG. 1.—Transverse section of outer growth layers of old stem, showing arc
of traumatic "resin" cysts induced by feeding of *Pissodes dubius;* first feeding of
budworm in 1918; ×15.

FIG. 2.—Transverse section, showing scar due to feeding of *Pissodes dubius;*
×25.

FIG. 3.—Portion of transverse section, cut at level (*a*) (text fig. 2); first
feeding of budworm in 1914; ×15.

FIG. 4.—Transverse section, cut at level *a* (text fig. 1); first feeding of
budworm in 1914; ×5.

FIG. 5.—Transverse section of wood in close proximity to pith, showing
diffusely scattered, dark parenchymatous elements; ×30.

FIG. 6.—Transverse section of 1912 and of portions of 1911 and 1913 growth
layers, showing zones of aggregated parenchymatous elements and "resin"
cysts; first year of feeding of budworm 1911; ×30.

FIG. 7.—Transverse section of portions of 1911 and 1912 growth layers,
showing zones of aggregated parenchymatous cells and "resin" cysts; first year
of feeding of budworm 1911; ×30.

FIG. 8.—Transverse section of wood, showing traumatic "resin" cysts induced by feeding of *Pissodes dubius;* ×40.

FIG. 9.—Transverse section of outer boundary of growth layer, showing loosely aggregated parenchymatous elements; ×50.

FIG. 10.—Transverse section of 1914 and 1915 growth layers, showing zones of aggregated parenchymatous elements and "resin" cysts; first feeding of budworm in 1914; frost injury at *f;* ×15.

FIG. 11.—Transverse section of 1916 and 1917 growth layers, showing zone of aggregated parenchymatous elements which appears to divide the 1917 ring into two distinct layers; ×30.

FIG. 12.—Transverse section of 1911, 1912, and 1913 growth layers, showing distribution of parenchymatous elements and "resin" cysts; first year of feeding of budworm 1911; ×25.

FIG. 13.—Tangential longitudinal section, showing various forms of amber colored substance in unsegmented wood parenchyma; ×65.

FIG. 14.—Tangential longitudinal section, showing various forms of amber colored substance in unsegmented wood parenchyma; note presence of septate tracheids; ×87.

FIG. 15.—Tangential longitudinal section, showing septate and non-septate wood parenchyma; ×120.

FIG. 16.—Tangential longitudinal section of tissue in close proximity to "resin" cyst, showing parenchymatous cells and septate tracheids; ×120.

FIG. 17.—Radial longitudinal section: *a,* tracheid with large bordered pits; *b,* unsegmented parenchymatous element with simple pits; *c,* similar cell in which amber colored substance forms tenuous transverse septa; ×400.

1

1918

2

3

1918

1916

1915

4

1914

1914

PLATE XIV

13

14

15

16

17
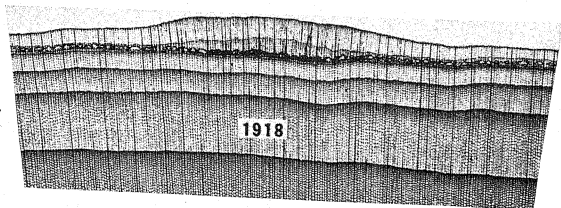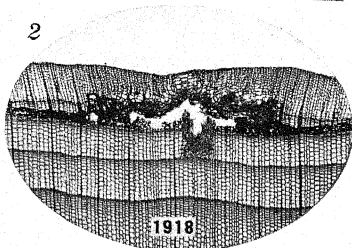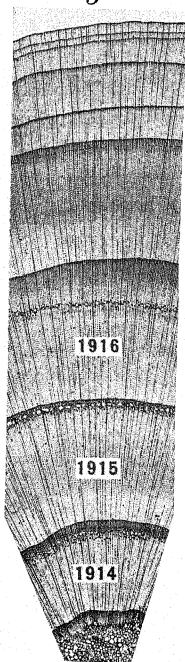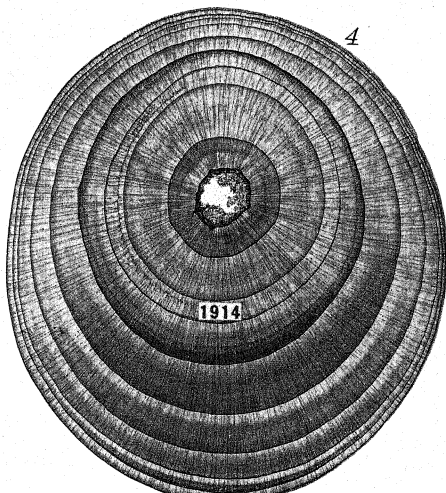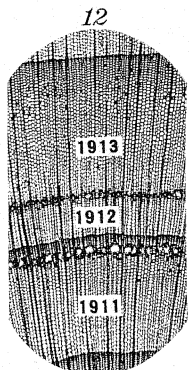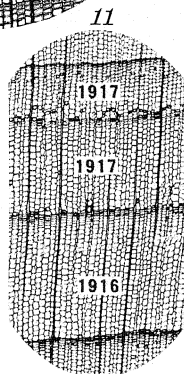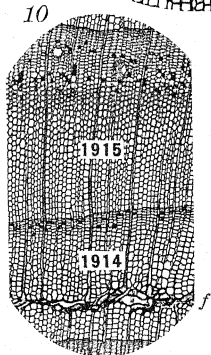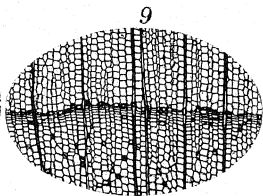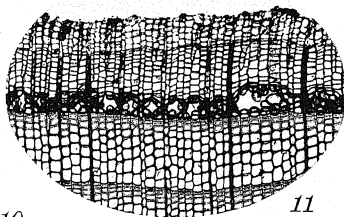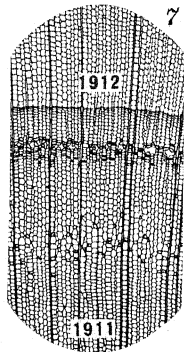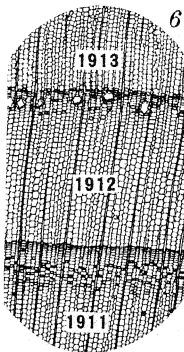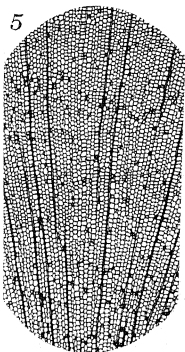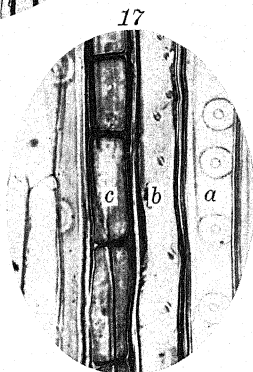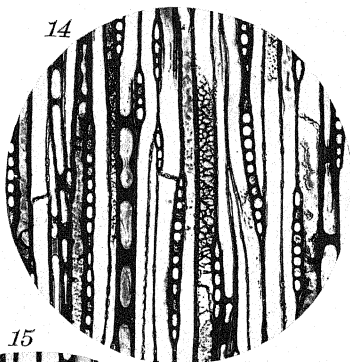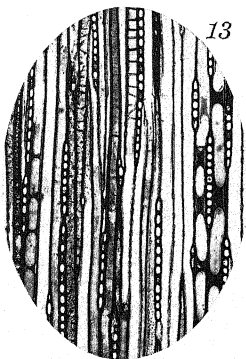
# MORPHOLOGY OF CONIDIOBOLUS VILLOSUS

## G. W. MARTIN

### (WITH PLATE XVI AND THREE FIGURES)

The fungus described in this note appeared in July, 1923, on a plate of nutrient agar inoculated from a piece of very rotten wood. This wood, collected in a moist thicket on the banks of the Iowa River, was covered with a brown *Hypochnus*, and an attempt was made, to secure the latter in pure culture. Other fungi, especially the one here considered, grew so rapidly in the cultures that the attempt was abandoned, and the original collection discarded, before it was realized that the most conspicuous contamination was an undescribed species of *Conidiobolus*. It was readily secured in pure culture, and proved itself capable of developing an extensive mycelium with great rapidity on ordinary nutrient media. On solid media the hyphae are of rather uniform size, averaging 12 $\mu$ in diameter and branching rather sparingly. When growing in a liquid medium, variation in size is greater and branching may be profuse. In either instance considerable difference in this respect may be due to the medium. Hyphae immersed in prune decoction, for example, branch much less freely than those growing in a decoction made from fresh green beans (text fig. 1). Growth is vigorous in both instances. As the hyphae grow, the older parts tend to become emptied of protoplasm, and cut off by septa from the protoplasm-filled tip. Frequently a septum appears before the protoplasm is all withdrawn, and in this way numerous segments are isolated, filled with protoplasm, with emptied and shrunken hyphal portions on either side (fig. 8 *c*). If food is abundant, these segments may send out lateral branches almost at once (fig. 20). In old and exhausted or dried cultures they tend to remain dormant and to accumulate, assuming various enlarged shapes, and forming hyphal bodies entirely homologous with the well known structures to which that name is applied, formed by the Entomophthoraceae attacking insects (fig. 19 *a-f*). In the species under consideration, however, these hyphal bodies

for a long time retain something of their mycelial character, to be explained, presumably, by the saprophytic habit.

As soon as the mycelium is well established, numerous conidiophores arise from the substratum. These differ from the hyphae of the mycelium mainly in their erect, aërial habit, and in their strongly positive phototropic reaction. As a rule, a slight and usually one-sided swelling develops in the conidiophore shortly before the discharge of the conidium (text fig. 2). The conidiophore is short when
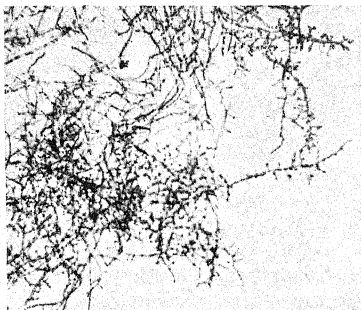


FIG. 1.—Photomicrograph of mycelium developed in bean decoction

growing normally, rarely rising into the air more than twice the diameter of the conidium.

In the formation of the conidium, the protoplasm of the conidiophore flows rapidly into the tip, which swells into a globose mass. When nearly all of the protoplasm has passed into the globose tip, the latter is cut off by a septum. The conidiophore remains turgid, its tip penetrating a short distance into the conidium as a dome-shaped columella. No change occurs for a while, but the protoplasm within the conidium may be observed to be in violent commotion. After some minutes the part of the conidium in contact with the columella is suddenly inverted, forming a short, blunt papilla which presses against the columella; the outer membrane surrounding both conidiophore and conidium is torn, and the latter violently dis-

charged. The maximum vertical discharge observed is over 25 and less than 30 mm., while the lateral distance which the conidia may be thrown, toward the light, is slightly over 30 mm. After the discharge of the conidium, the conidiophore remains turgid for a few minutes, the columella appearing as a swollen tip, with traces of the broken membrane apparent at its base, and either with or without a minute apiculus at its tip (text fig. 2 *b*, *d*, *f*). After a few minutes the conidiophore begins to collapse, and 15 or 20 minutes after the discharge it is shrunken and flaccid (text fig. 1 *e*). The collapse is gradual, not sudden as described by BULLER (2) in the
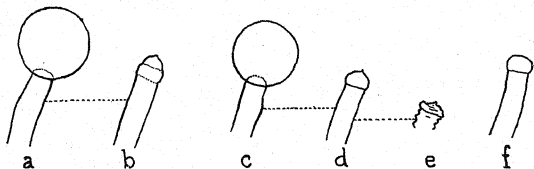


FIG. 2.—Discharge of conidia: *a*, *b*, same conidiophore before and after discharge; *c*, *d*, second type of conidiophore; *e*, collapse of conidiophore shown in *d*, 15 minutes later; *f*, conidiophore without apiculus; all growing in natural position in air on nutrient agar in slide culture.

case of the basidium of an agaric. But one conidium is borne on a given conidiophore.

This account of the discharge of the conidia, based on repeated observation of living material, agrees substantially with the statements concerning the discharge of similar spores, as made by BREFELD (1) and OLIVE (4). BREFELD, referring to *Conidiobolus utriculosus*, mentions the turgidity of the conidiophore, and describes the discharge as accompanied by a sudden wrench. OLIVE, discussing *Empusa*, particularly *E. sciarae*, basing his account on stained sections, states that "the penultimate cell" (the conidiophore) "forms the explosive mechanism." This is scarcely the case in the species here described. It is true that the conidiophore remains turgid and swollen up to and after the discharge of the conidium, but the active mechanism is the sudden reversal of the basal papilla, due to increasing pressure within the conidium itself.

At room temperature, the conidia are produced in great abund-
ance within 48 hours after a culture is started. They are globose,
with the prominent basal papilla characteristic of the family, very
variable in size, ranging 12–46 $\mu$ in diameter, and averaging about
32 $\mu$ (figs. 1, 2; text fig. 3). The smaller dimensions are in general to
be attributed to conidia of secondary, tertiary, or a higher order, but
direct measurements (made by means of a horizontal microscope)
of conidia growing naturally in a culture slide just before their dis-
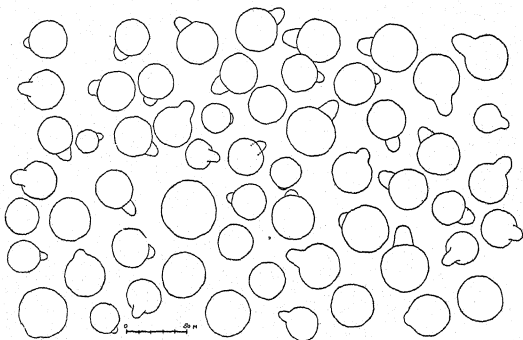


FIG. 3.—Fifty-seven conidia from 42-hour culture on synthetic agar, outlined with
camera lucida to show variation in size.

charge average only very slightly higher. Traces of the torn outer
membrane may sometimes be observed as a narrow collar at the
base of the papilla. If conditions are favorable, germination may
take place at once, and may result in the formation of a mycelium
or of a secondary conidium. In the latter case, a germ tube emerges
laterally from the conidium, and, after attaining a variable but
usually short length, swells at the tip. This swollen tip is cut off as a
conidium and discharged in the usual way. The process of germina-
tion and formation of a secondary conidium has been followed in
water and in prune decoction mounts, and has been found in a typical
case to require about 5 hours for its completion (figs. 1–7). If the

spore is deeply immersed, the germ tube may be very long and more
or less branched. In such case the protoplasm is progressively with-
drawn from the basal parts of the hypha (fig. 8).

After a culture is a few days old, spores begin to appear which
differ from conidia in the possession of numerous cylindrical out-
growths, soft, blunt, and hairlike as a rule; in the thicker wall and
darker color of the cell contents, and in the substitituon of an opaque,
pluglike structure for the papilla (figs. 12–15). In other respects
these spores are exactly like the conidia, and since there may always
be found in such cultures a complete series of intermediate stages
(fig. 11), the obvious conclusion is that they develop from ordinary
conidia and serve as resting spores. A few resting spores have been
seen in which the hairs were slightly swollen at the tips. In one
culture a very few of the resting spores were provided with distinctly
conical, spinelike processes. Intermediate stages were present in all
cases, and such aberrant spores seem to represent minor variations
due to unknown causes.

In the living fungus the conidia and mycelial parts are filled with
a mass of dense, refractive globules. These do not seem to be fatty
in nature; at least, they blacken very slowly with osmic acid and
do not stain with Sudan III. Because of their presence, no nuclear
structures can be seen, either in the conidia or the mycelium. Sec-
tions cut $5\ \mu$ thick and stained with iron-alum haematoxylin show
numerous very small nuclei present. These are spherical or some-
what elongated, and about $1.5\ \mu$ in diameter. As is to be expected,
they are most numerous in the growing tips of hypae and in the
conidia (figs. 20–24). In conidia which have become more or less
dormant, the nuclei tend to be distributed near the periphery (fig.
21). The nuclei of the resting cells seem to differ in no respect from
those of the conidia (fig. 24). No recognizable division stages of the
nuclei have been observed. No traces of conjugation nor of
zygospores have been seen.

The occurrence of spores with a roughened or sculptured outer
wall is exceptional, but not unknown in the Entomophthoraceae.
THAXTER (6) describes the zygospores of *Empusa echinospora* as
spinose, and SPEARE (5) has shown that in *Massospora cicadina* the
conidia are verrucose and the resting spores are strikingly reticulate.

BREFELD describes the zygospores of *Conidiobolus utriculosus* as beset with small warts. The resting spores of the species under consideration are quite unlike any of these, however, and in the literature there seems to be no suggestion of the peculiar transformation of conidia into resting spores here described.

The fungus clearly belongs to the genus *Conidiobolus* established by BREFELD to include two species which he found occurring as parasites on species of *Hirneola* and *Exidea*. One species, *C. utriculosus*, is described in detail. BREFELD's second species, *C. minor*, is based on certain small conidia observed in the cultures of *C. utriculosus*. It was not secured in pure culture, and it seems highly probable that *C. minor* represents small conidia of *C. utriculosus*, reduced in size by repeated germination in exhausted cultures. Similar reduced conidia occur in the case of the species under consideration, when it is growing under such conditions (fig. 25). The species found in Iowa closely resembles *C. utriculosus* in its vegetative and conidial characters. It differs, however, in the size and shape of the conidia, which in *C. utriculosus* are oval and average $35 \times 50$ $\mu$ in size, and very markedly in the possession of the curious hairlike appendages on the resting spores. No additional species of *Conidiobolus* seem to have been reported since BREFELD's original publication. In 1919 GILBERT (3) reported his observations upon a saprophytic Entomophthorous fungus, which he isolated from fern prothallia, without, however, suggesting the genus to which it belonged. There are a number of discrepancies between his observations and those reported in this paper. Thus the primary conidia of the Wisconsin fungus are described as varying from 48 to 60 $\mu$ in diameter, as compared with an average of slightly over 32 $\mu$ for the form studied in this laboratory. GILBERT found no hyphal bodies; they are abundant in old cultures of the Iowa form. His description of the conidiophores and of the discharge of the conidia is in general agreement with the observations made here when germination takes place under the artificial conditions prevailing in a liquid mount, but does not agree with the observations made upon the Iowa fungus when growing naturally in a slide culture chamber and studied with a horizontal microscope. Under such conditions, the conidiophore is

only very slightly swollen, the inversion of the papilla and the dis-
charge of the conidium are practically simultaneous, the one being
the cause of the other, and no portion of the contents of the conidio-
phore has ever been observed to be discharged with the conidium.
GILBERT found old conidia in his cultures becoming yellowish, with
a slight thickening of the spore wall, but makes no mention of the
curious and striking appendages, nor does he refer to the most strik-
ing characteristic of the Iowa form, namely, the regular and gradual
transformation of the conidia into the appendaged resting spores.
In spite of these discrepancies, it seems probable that the Wisconsin
fungus and the one isolated in Iowa represent the same species.
While the resting spores are distinctly unusual, there would seem to
be little warrant for regarding this character as justifying the estab-
lishment of a new genus, when the fungus is clearly related very
closely to *Conidiobolus utriculosus* as described by BREFELD. It is
therefore described as a new species of the same genus, the specific
name assigned to it referring to the villose appendages of the resting
spores. The formal diagnosis is as follows:

    **Conidiobolus villosus,** n. sp.—Mycelium abundant, saprophytic,
coenocytic, becoming septate and fragmented; hyphae immersed
in substratum usually richly branched; aërial hyphae sparingly
branched, averaging 12 $\mu$ in diameter; conidiophores erect, positively
phototropic, not strongly differentiated from mycelium; conidia
globose, with large basal papilla, 12 to 46 $\mu$ in diameter, averaging
32 $\mu$; conidia on germination give rise either to mycelium or to
secondary conidia borne on short conidiophores; under conditions
unfavorable for germination, conidia become gradually transformed
into resting spores, characterized by thicker walls and darker color,
by the contraction of the papilla into a pluglike base, and by the
development of numerous soft, blunt, hairlike appendages; germina-
tion of resting spores like that of conidia; hyphal bodies abundant in
substratum; zygospores unknown.

    On *Hypochnus* sp. on sodden, decayed, frondose wood. Iowa City, Iowa,
July 1923; also Wisconsin (?).

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      IOWA CITY

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## EXPLANATION OF PLATE XVI

All figures drawn with aid of camera lucida, using Zeiss objective D and ocular 4; reduced about one-third in reproduction.

FIGS. 1–7.—Germination of conidia in prune decoction (figs. 5–7 represent same conidium): fig. 1, conidium from old culture freshly placed in solution; fig. 2, after 2 hours, vacuoles indistinct; fig. 3, germ tube almost complete, 3.5 hours; fig. 4, secondary conidium half formed, 4 hours; fig. 5, old spore almost emptied of protoplasm, 5.5 hours; fig. 6, secondary conidium discharged, conidiophore still turgid, 5 hours, 50 minutes; fig. 7, conidiophore and old conidium wall collapsed, 6 hours.

FIG. 8.—Germinated conidium, 17 hours in prune decoction on slide, showing septate and collapsed tube (over 1500 μ long) with segregated hyphal body, and secondary conidium.

FIGS. 9, 10.—Two conidia with multiple germ tubes, germinated on dry slide in moist chamber.

FIG. 11.—Conidium becoming transformed into resting spore; 15 day culture on agar plate; note fragment of conidiophore wall.

FIG. 12.—Newly formed resting spore, from same culture as fig. 11.

FIGS. 13–15.—Resting spores from surface of 9-weeks' prune decoction culture: fig. 13, hairs more numerous and longer than usual; figs. 14, 15, resting spores shrunken to oval shape, and with papillae transformed into pluglike bodies.

FIGS. 16–18.—Germination of resting spore: fig. 16, secondary conidium nearly formed; fig. 17, conidium ready for discharge, 12 minutes later; fig. 18, conidium discharged, 18 minutes later than fig. 17.

FIG. 19.—a–f, Hyphal bodies from 2-months' culture on nutrient agar.

FIGS. 20–24.—Stained sections, to show nuclei: fig. 20, hyphal segment germinating in agar culture, growth toward right; fig. 21, conidium in dormant state; figs. 22, 23, production of secondary conidia; fig. 24, resting spore.

FIG. 25.—Smallest conidium observed, diameter 12 μ.

PLATE XV.
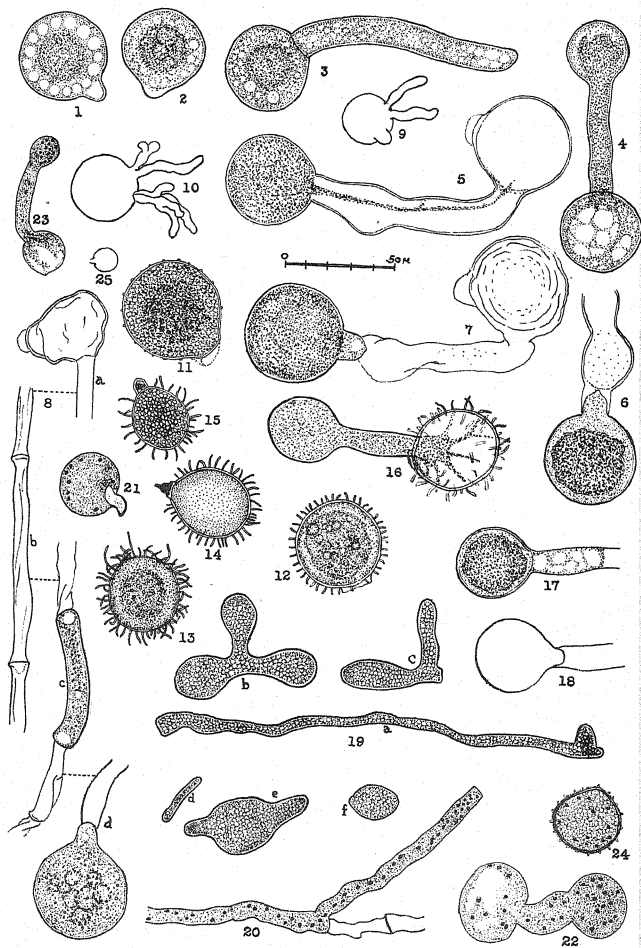
# POISON CANALS OF CICUTA MACULATA

## H. B. SIFTON

### (WITH PLATES XVII, XVIII)

The points illustrated in this paper were brought out during a microscopic examination of *Cicuta maculata* L., made with a view to the identification of fragments of the plant in cases of poisoning, and have been thought sufficiently interesting to be worthy of record.

The secretory canals of the Umbelliferae, running in a longitudinal direction in all the vegetative organs, are rather easily observable structures, and their presence has been known for many years. As early as 1866, MÜLLER (4) described the canals in the secondary tissues of the root as schizogenous structures increasing in size with increasing age, but with little or no multiplication of the cells forming their walls. His figures are from *Ferula orientalis*. DEBARY (2) has described minutely their development in the primary cortex.

In the present article, some details observable in the root will first be described. Fig. 1 is from a transverse section of one of the fleshy roots, in which a considerable amount of secondary growth has taken place. The dark line of small cells near the right is the cambium, with the secondary cortex to the left. The tissue produced by the cambium is chiefly parenchyma, and on the cortical side secretory canals are plentiful in this secondary growth. The identity of the cells that are to line the canals is plain at a very early stage, seven cells from the cambium in the youngest canal shown in the photograph. They lie in two rows adjacent to each other, and are to be distinguished by their dark contents. The development of the canal consists largely in the spreading apart of these rows of cells.

One of the young canals in its earliest stage is shown at a higher magnification in fig. 5. The two rows of lining cells are very distinct, almost in contact, but with a narrow slit forming between them at one end. In size they differ considerably from the surrounding parenchyma cells, and it is clear that either the cambium cells which

gave rise to them divided more rapidly than those on either side, or divisions have since taken place in the canal cells themselves. The parenchyma cells in the immediate neighborhood of the canal are gorged with starch. When a root section is treated with iodine, the accumulation of starch around the younger canals is very striking. As the canals increase in size the starch gradually disappears, being used doubtless in the manufacture of the secretion with which the structures are filled. Around the large, fully formed canals starch is no more plentiful than anywhere else in the cortex.

Figs. 6 and 7 show further stages in development of the secretory canal. Fig. 6, taken at a greater distance from the cambium than fig. 5, shows the slit opened out until the canal is lenticular in cross-section. The only additional change is an increase in size in all the cells. This increase is typical of that found throughout the cortex; the outer cells, with the exception of the periderm, are always larger than the younger ones near the cambium. Fig. 7 shows a completed canal such as may be seen to the left of fig. 1. Its cross-section has become more or less circular, and some of the secretion, with which it was originally filled, has been retained through the process of imbedding, cutting, and staining. The lining cells have become much elongated. The drawing away of the protoplasm from the walls of the cells is due to imperfect fixing and imbedding.

Fig. 8 is of a longitudinal section through one side of a canal, and shows the secretory cells in face view. The arrangement of the cells in vertical rows and their shortness in a vertical direction suggest that horizontal divisions have occurred in the process of their formation. This is strengthened by the much greater length of the adjacent parenchyma cells formed by the same cambium. Cells in the process of division, however, have not been found. Outside the area shown in fig. 1 are the canals of the primary cortex as described by DeBary. A few of them are shown at the right of fig. 2. They resemble closely the fully formed secondary canals just described. When the root is young they are small, but their size increases with age and they also are filled with secretion.

The region of the root inside the cambium contains no canals, a fact of some interest in connection with the varying toxicity of this organ at different seasons of the year. The poisonous principle is

contained in the canals, from which it oozes in the yellowish drops
that appear when a fresh root is cut. Much has been written about
*Cicuta* from the standpoint of its toxic qualities, and widely differing
opinions as to its toxicity have been recorded. It is rather well
agreed, however, that the roots are more poisonous in spring and
autumn than in summer. JACOBSON (3) states:

> Cicutoxin [the poison] is very sensitive to an increase in the temperature
> above that normal to its formation in the plant, and differences have been ob-
> served in the products obtained in April and October from those obtained in
> July and August. The former is the yellower product and the more toxic, grain
> for grain, but the midsummer tubers contain the larger quantity of material.

From his experiments it would seem that this is due to polymeriza-
tion and perhaps partial decomposition at the higher temperature.
In addition to this, there is another reason why the bulky roots found
in summer are less toxic than might be expected, in that they have
a large central region devoid of poison canals. This region is being
added to not only by the cambium of the root, but also by divisions
and growth in the parenchyma cells of the primary tissues. This
growth is illustrated by figs. 2 and 3. Fig. 2 represents a transverse
section of the central part of a comparatively small root. The cam-
bium has produced only a small amount of secondary tissue, and the
primary wood forms an irregularly star-shaped mass at the center.
Fig. 3, from a section of a larger root, shows the primary wood
forced apart by the growth and division of the parenchyma cells.
The two figures are at the same magnification, but in that of the
larger root even the radial rows of cells formed by the cambium on
its inner side are beyond the range of the photograph, owing to the
extent of the growth of central tissue. In spring none but small roots
are to be found, and in autumn the large ones have been reduced to
mere withered remnants, all their substance apparently having been
used up in the process of seed production. At this time the only
fleshy roots to be found are the small ones belonging to the seedlings
whose first crop of seeds will not be borne until next year.

To determine whether cicutoxin might be present in the central
portion of the root, although canals are absent, the chemical test
recommended by TAKAYAMA (6) was used, which is quoted by
JACOBSON as being efficient in determining the presence either of the

poison itself or of its polymerized and decomposed product. Crushed portions of the root were soaked in a mixture of three parts of phenol to one of ether, and a drop of concentrated sulphuric acid was brought into contact with the solution. This test, when performed on the parts of the root outside the cambium, invariably gave the green color, changing through blue and violet to brown, that is characteristic for cicutoxin and its derivatives, but the result was never obtained when the central part of the root was tested.

The rootstock differs from the root in having a pith, and in the enormous size attained by the secretory canals. Fig. 4 is from the cortical portion of the rootstock, and is at the same magnification as figs. 1-3. The right of the photograph is toward the cambium. The large canals with distorted and torn parenchyma between them are prominent in the figure, and a few smaller ones are to be seen nearer the cambium. The pith also contains canals, but these have not been illustrated. Like those of the cortex, they grow to a size unequaled either in the root or in the aerial portions of the plant.

The stem proper has no canals in its secondary tissues. In its cortex a single primary canal is opposite each of the larger fibro-vascular bundles. These canals do not grow with the increasing size of the stem, but on the contrary often appear flattened as if crushed by the growing tissues. Fig. 9 shows one of the fibro-vascular bundles of a stem where a great deal of secondary growth has taken place. Opposite the crown of primary bast, and separated from it by two layers of cortical parenchyma, is one of the small, flattened canals whose lumen is almost eliminated, although the dark walled lining cells are quite clear.

Like the rootstock, the aerial stem has canals in its pith. In a stem the size of that from which fig. 9 was made, the pith is hollow except at the nodes, owing to the breaking down of its central cells. Imbedded in the remaining peripheral tissue are a few small canals. Although there is an abundance of room for growth, owing to the breaking down of most of the medullary tissue, these canals do not enlarge, but remain like those of the stem cortex in size. They, however, do not attain the flattened appearance that has been described for the latter.

Accompanying the vascular bundles through the petioles to the leaves are small canals similar to those in the stem, with which they probably connect, although a determination of this connection was not made. The large ducts in the fruits are too well known to require more than a passing mention.

It should be noted that the scarcity and small size of canals in the stem and leaves, as compared with those of the root and root-stock, correspond with the comparative lack of toxicity of the former organs. Numerous deaths from poisoning by the underground parts of the plant are on record, the action being very rapid, even in cases where cooking may be held to have destroyed a great deal of the cicutoxin originally present. Moreover, the amount necessary to produce death is very small. On the contrary, cases of death from the stems and leaves are very rare. PAMMEL (5) states a case where some cattle and a horse were made ill, but not killed by eating leaves of the plant in hay. JACOBSON quotes from CAILLARD (1) the case of a man who obtained and ate large quantities of the young stems and leaves of *Cicuta virosa* in an attempt at suicide. Although very ill, he responded to treatment and recovered. JACOBSON states:

Experiments both in this laboratory and elsewhere have brought out the fact that the dried stems and leaves of the *Cicuta occidentalis* class have not been found to be poisonous except in the early spring, and then the cicutoxin is present only in minute quantities.

The explanation of this is made clear by the anatomical study of the plants.

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## DESCRIPTION OF PLATES XVII, XVIII

*PLATE XVII*

FIG. 1.—Transverse of mature fleshy root; cambium at right, with second-ary cortex to left; ×48.

FIG. 2.—Transverse of central portion of young root; ×48.

FIG. 3.—Transverse of central portion of larger root; ×48.

*PLATE XVIII*

In all figures of this plate, right of photograph is toward central axis of plant.

FIG. 4.—Transverse of rootstock; many large canals; ×48.

FIG. 5.—Transverse of secondary cortex of root near cambium; canal just beginning to open; ×307.

FIG. 6.—Transverse of same farther out from cambium; canal midway in its development; ×307.

FIG. 7.—Transverse of same still farther from cambium; completely formed secondary canal; ×307.

FIG. 8.—Radial of same; wall of canal in face view; ×307.

FIG. 9.—Transverse of stem; bast and secondary wood of one fibrovascular bundle; collapsed primary cortical canal to left of crown of phloem; ×103.

1



2
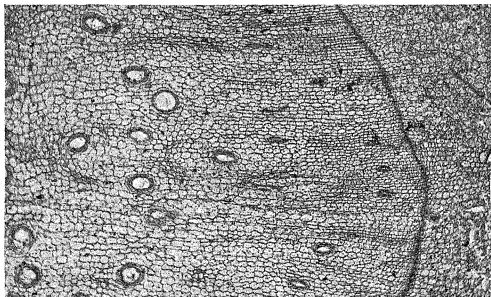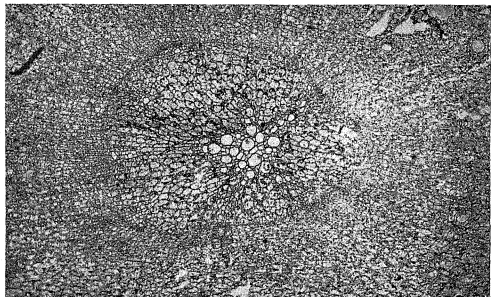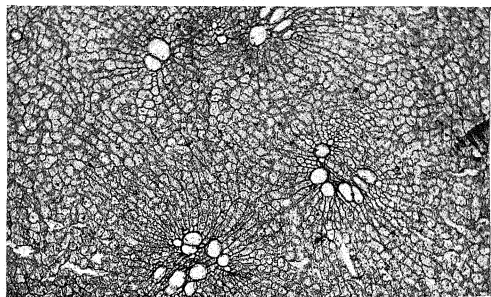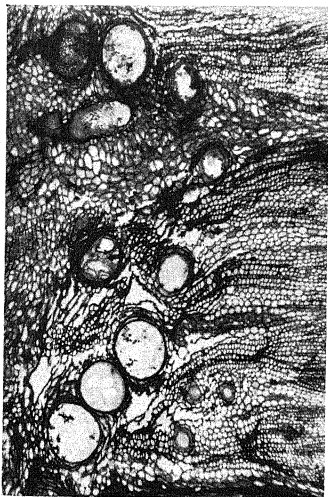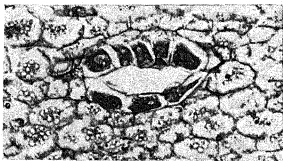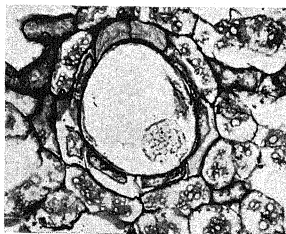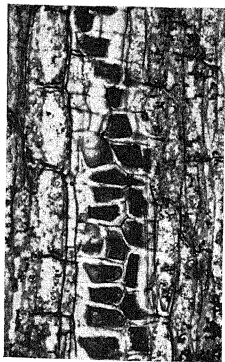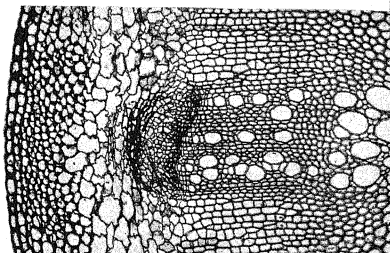
# THE PECTINASE OF SCLEROTINIA CINEREA

GEORGE W. MUHLEMAN

## Introduction

*Sclerotinia cinerea* attacks certain species of non-resistant plums. It is commonly called the brown rot fungus because of the color which seems to develop after the fungus has attained a certain growth. The infected plum turns brown, but is quite solid, even when well rotted. The term *Sclerotinia*, therefore, indicates sclerosis or hardening, and *cinerea* refers to its cinder-like appearance. The writer has observed that when the fungus has been allowed to grow three or four days on prune juice it has a grey color, and so remains until the mycelium has been ground up, when the oxidases cause it to turn to colors ranging from a very light to a dark chocolate color.

When the mycelium is observed under the microscope, it is found to be segmented in appearance, similar to *Spirogyra*. The spores are greatly branched, and have the appearance of the yeast plant after it has budded and branched.

The spores when formed in great profusion have a rust colored appearance, and seem to arrange themselves in a series of circular areas. The right conditions for the formation of spores has been one of the quests of this investigation, and will receive further attention as this investigation proceeds. The fungus seems to be rather erratic; sometimes the spores are best formed in the dark and again they are best formed in the sunlight. This investigation, however, has demonstrated that a nutrient medium can be inoculated either with the spore or by introducing a filament of the mycelium, a convenience if one has not always on hand a supply of spores for inoculating purposes. Nevertheless, the method of inoculating by dusting in the spores is by far the more satisfactory, because of the uniformity with which the spores become distributed over the surface. Usually from thirty inoculations the growth was quite uniform, except in the case of possibly three or four flasks.

The fungus was grown in an incubator cooled by passing tap water through the jacket, and also by placing the Erlenmeyer

flasks containing the cultures on top of the laboratory table and
in the trough of the table. Some of the flasks were covered and some
were not. The room temperature was 26° C. The control conditions
of light and temperature seemed to make no appreciable difference
in the growth of the fungus or in the production of spores, hence the
flasks containing the nutrient prune juice after inoculation were
simply placed on top of the laboratory table. Sporulation of
*Sclerotinia cinerea* grown on prune juice occurred at from 5 to 13
days, and in other cases spores never formed. Peaches that had
been soaked in distilled water for 24 or 48 hours, and then auto-
claved and inoculated, in some cases produced abundant spores in
3 days, while some peaches inoculated at the same time never
produced spores. If, instead of soaking the peaches 24 hours before
autoclaving and inoculating, one simply placed dry peaches in
Petri dishes containing distilled water, and placed them in the
autoclave until the peaches had softened, these could not be inocu-
lated at all, presumably because the peaches had been bleached with
$SO_2$, and the presence of the $SO_3$ or $SO_4$ radical inhibited all fungus
growth.

After growing a crop on prune juice, attempts to grow a second
crop on the same juice resulted in a much slower growth, as well as
a smaller yield. The color of the prune juice was made very dark
as a result of one growth. There did not seem to be a very great
decrease in the volume of the prune juice after the growth of the
fungus. These observations are to be continued.

The peaches when placed in Petri dishes and sterilized became
infected by one of the black fungi if allowed to stand for a period.

An attempt was made to grow *Sclerotinia cinerea* on a peach that
had been soaked and then mashed out flat on the bottom of the
Petri dish and sterilized. No growth was possible. An attempt to
grow this fungus on nutrient agar spread out on the bottom of the
Petri dish was also only partially successful.

The specimens used in this study were secured from the plant
pathology laboratory of the University of Minnesota, from a
"mummy" found under a plum tree in the plum orchard of the
Farm School of the University, and from an infected plum found
in a plum orchard near Mora, Minnesota.

The best sporulation was secured when the fungus was grown on peaches. Prune juice was used for the growth of the felts from which the pectinase was secured.

The method of preparing the peaches and the prune juice was to place the dry peaches and dry prunes in distilled water, and keep in the icebox 24–48 hours. At the end of this time both the peaches and the prunes were nicely swollen and soft. The prunes used were a large meaty variety. The peaches were removed from the liquor, and one or two halves were placed in a Petri dish and autoclaved for 30 minutes at a pressure of 15 pounds. After they were cooled they were inoculated from the spores by dipping a coil of sterile wire on to the spores, and then dusting them on to the peaches. Perceptible growth of the spores took place at room temperature in from 2 to 3 days, and at the end of 5 days in most cases sporulation had occurred.

The prune juice was prepared by placing the large beaker, in which the prunes had been allowed to soak, in the autoclave and cooking 20–30 minutes at 15 pounds pressure. The entire contents were then poured into the basket of a hand press lined with a heavy cloth, and subjected to pressure. The pits were then removed from the prune pulp and the pulp placed in more distilled water and again autoclaved. This process of expressing the juice and cooking the pulp in the autoclave was continued only so long as a juice of specific gravity 1.03 was secured. The juice thus secured was thoroughly mixed, and the specific gravity determined and marked on the outside of the flask. About 250 cc. was placed in Erlenmeyer flasks which were stoppered with cotton, and the contents autoclaved for 15 minutes at 15 pounds pressure. As juice was needed from time to time, it was taken from these flasks and either diluted to the specific gravity desired or used as it was prepared.

There was placed in a series of 300 cc. Erlenmeyer flasks 20 cc. of prune juice, a volume sufficient to cover the bottom of the flask; the flasks were stoppered with plugs of cotton, and autoclaved for 15 minutes at 15 pounds pressure. In the first attempts at inoculating, the sterilized loop of wire, after touching the spores, was dipped beneath the surface of the prune juice. No growth of the fungus resulted from this method of inoculating. In every case when the

wire laden with spores was tapped on the lip of the flask, the spores thus dusted on to the prune juice grew in great abundance.

To prepare an active pectinase solution, three different methods of procedure were employed. (1) The method outlined by BROWN.[1] After the mycelium had grown sufficiently, the felts were removed from the flasks, washed in water to remove all the liquor, and then washed twice in acetone. These washed felts were then exposed to the air to allow the acetone to spontaneously evaporate, and afterward dried in a desiccator charged with fresh quicklime. This dry mycelium was then ground with an equal weight of silica and mixed with water in the ratio of 0.2 gm. of the ground substance to 3 cc. of distilled water. (2) The felts were removed from the prune juice in which they had grown, washed in running water until free from prune juice, dried between cloths, thoroughly ground in a mortar with silica, and placed in a hydraulic press and subjected to a pressure of 400 kg. per sq. cm. for 30 minutes. The juice thus secured showed a marked activity. (3) The felts were removed from the culture media, thoroughly washed in running water, dried between cloths, ground up in a mortar with silica, and then triturated with enough water to make a mixture of the same limpidity as that prepared under the first method.

By repeated experimentation it was found that the average weight of the dry felts grown on 20 cc. of prune juice in 5 days was about 0.18 gm.; therefore to determine the weight of silica to be added to the wet felts, the number of flasks was multiplied by 0.18. The last method of preparing a macerating solution was the simplest, and the one involving the least expenditure of energy. The task which is to engage my attention later is to prepare a solution that will be standard in its concentration of enzymes.

HARTER and WEIMER reported that, in their work with certain species of *Rhizopus*, they secured an active pectinase solution by using the media in which they grew their fungus. In no case has the writer been able to secure any evidence of the pectinase enzyme in the prune juice media after growing a crop of *Sclerotinia cinerea*. The evidence of maceration was tested out on green plums, green

    [1] BROWN, W., Studies in the physiology of parasitism. Ann. Botany 29:313–348. 1915.

apples, green peaches, and ripe potatoes cut in sections 0.5 mm. thick. An attempt was made also to precipitate the enzyme from solution by using ethyl alcohol and purifying in the usual way. Here again negative results were secured.

With an active pectinase preparation, the disks were macerated 1.5–4 hours. These disks were sectioned with an ordinary microtome set to cut 0.5 mm. thick. The apple and plum disks were reduced to a sauce and the potatoes to a fine pulp. The disks when cut, even after remaining in water for a number of days, were very resistant to being pulled apart, but after maceration they were very easily separated by lightly holding between the thumb and forefinger. This was the usual method for testing maceration.

The pectinase solution was preserved in toluene, and the maceration was carried on with toluene added as a preservative, the amount added being just sufficient to give to the mixture a strong odor of the preservative. Later studies may require a resort to quantitative methods of procedure. The macerating solutions thus preserved have retained their activity from one week to three months. When a standard solution has been prepared, perhaps there will be no variation as to the time the solution remains active. It was found that 10 or 12 felts would provide material to prepare the necessary macerating media. In grinding the mycelia one always stopped when the pulverized mass no longer adhered to the pestle or to the mortar. An attempt to use a meat grinder to hasten the work of grinding was found impracticable, as the material clung to the chopper and clogged it.

After it was determined that Sclerotinia cinerea of 3, 4, or 5 days' growth gave the most active pectinase solution, it was further discovered that it could be ground up in a mortar in a few minutes. The color of the felts and the color of the ground material, and not exactly the age of the crop in days or hours, must govern one's conclusion as to its activity. In every case of the score or more active preparations made, the color of the ground mass was a light chocolate. The longer the fungus was allowed to grow the tougher and thicker became the felt. Attempts to secure an active pectinase solution from old growths were failures.

To determine whether the specific gravity of the prune juice

has any influence on the rapidity of the growth of *Sclerotinia cinerea*, duplicates of three flasks were inoculated with the following concentrations of prune juice: specific gravity 1.04, 1.05, 1.06, 1.07, 1.08, 1.09, 1.10, 1.11. These were all inoculated from spores grown on autoclaved peaches. There did not seem to be any perceptible difference as to the amount of growth. The relative macerating activity of the enzyme from these different cultures will be taken up in later experimentation. The heaviest spore growth seemed to have taken place on felts grown on prune juice with specific gravity 1.04, and this was the concentration employed in further studies.

## Summary

1. Spore formation and methods of inoculating with spores and with mycelium are discussed.

2. Methods of growing the fungus on different media are described.

3. One growth of fungus seems to unfit prune juice for growing further crops.

4. Methods of preparing culture media which give maximum growth are described.

5. Three different methods of preparing an active pectinase solution are compared.

6. *Sclerotinia cinerea* does not excrete pectinase into the culture media.

7. The disks used in testing maceration were 0.5 mm. thick, and were from apples, plums, peaches, and potatoes. The tensile strength was determined with the fingers.

8. It has been demonstrated that the color of the felts of *Sclerotinia cinerea* is a good index of the activity of the pectinase solution which may be prepared.

9. The specific gravity of prune juice has been studied.

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# POLYEMBRYONY IN CERTAIN POLYPODIACEAE AND OSMUNDACEAE

## DAVID M. MOTTIER

### (WITH THREE FIGURES)

During the years 1920 and 1921, a graduate student in the botanical laboratories of Indiana University carried out some experiments in the development of several embryos on single gametophytes of certain species of Polypodiaceae, the results of which have been published elsewhere.[1] ETTER'S experiments, which were necessarily brought abruptly to a close, not only gave promise of further positive results, but suggested also other lines of study. The writer, therefore, continued the study of the experimental development of polyembryony, and at the same time began a series of investigations of a similar character which are still in progress. In ETTER'S paper will be found a résumé of the more important literature available.

## Method

Stock cultures of the prothallia were first made, and, as soon as the plants had attained a width of 2–4 mm. across the wings, thrifty individuals were carefully transplanted to separate dishes and kept under the best cultural conditions available. Fig. 1 illustrates the method of cultivation. All cultures were made upon sterilized woods earth, in ordinary flower pot saucers, which were covered with bell-jars. The cultures were watered by sub-irrigation and never by sprinkling or spraying. In case the plants touched one another, or in any way became crowded because of increase in size, a number of the specimens were again transplanted to other dishes.

As soon as the prothallia had attained a diameter of 10–15 mm., fertilization was accomplished by hand. The time required for the attainment of this size varies with the season, the plants growing more rapidly during the spring and summer months. The record of the plants shown in fig. 1 is as follows: spores sown October 11,

[1] ETTER, AUSTIN, Polyembryony developed under experimental conditions in certain polypodiaceous ferns. Bull. Torr. Bot. Club 50:95–107. 1923.

transplanted December 30, 1922, and photographed (natural size)
February 28, 1923. These plants, therefore, were nearly four months
old. Needless to say, it was necessary to prevent fertilization dur-
ing this period by care in sub-irrigation, and by preventing the
falling of drops of water upon the plants.



Fig. 1.—Four months' old prothallia of *Matteuccia nodulosa* (Michx.) Fernald, grown
upon woods earth in flower pot saucer, showing method of cultivation; natural size.

To accomplish fecundation, a few drops of water were placed
upon the prothallia near the growing point. The best time was found
to be near midday during sunny weather. In prothallia that tend to
be dioecious, like those of *Matteuccia nodulosa* (Michx.) Fernald, a
small tuft of male prothallia was placed near the apical sinus before
the water was applied.

### Observations

In these studies the following species supplied the material: *Matteuccia nodulosa* (Michx.) Fernald, *Dryopteris mollis* (Jacq.) Hieron., of the Polyopdiaceae; and *Osmunda Claytoniana* L., of the Osmundaceae. *Pteris longifolia* L. is also a suitable plant because of the ease with which prothallia can be grown.

The process of fertilization was repeated two or more times on as many successive days. One of the objects was to determine how many self-sustaining sporophytes could be produced on a single prothallium before the latter began branching by offshoots or prolifera- tions. In *Dryopteris*, *Matteuccia*, and *Osmunda*, as many as five sporophytes could be brought to the stage of independent growth on a single prothallium before the latter began to show signs of exhaustion, which was indicated by the cessation of growth and gradual exhaustion. Three or four sporophytes, drawing at least a part of their nourishment from the parent gametophyte, soon ac- complished the exhaustion of the latter. In addition to the large, self-nourishing sporophytes, one or more embryos of microscopic size were sometimes detected. If, however, the first sporophytes were carefully amputated, a larger number could be produced. With some care, and after a little practice, a steady hand can amputate, or remove by plucking away, a sporophyte that has its first leaf and root large enough to be seized with the forceps. The amputa- tion is made flush with the under surface of the prothallium. A very sharp lance-pointed needle, a "razornife," or a safety razor blade provided with a handle is a suitable tool.

After the prothallia have attained a certain size and age they begin to develop branches or proliferations. This behavior was especially pronounced in certain specimens of *Dryopteris mollis*. The proliferations, generally heart-shaped, usually arise from the under surface on either side of the archegonial cushion, now devel- oped into a distinct midrib. Proliferations may also spring from the margins of the wings, and from both anterior and posterior portions. Even after the older portion of the midrib has become brown on the surface, proliferations will spring from the tissue beneath. The pro- liferations may be numerous, and each may develop a sporophyte

after fecundation. Fig. 2 shows a prothallium of *Dryopteris mollis*
with eleven heart-shaped proliferations, ten of which bear a rooted
sporophyte. These proliferations are all a part of the parent pro-
thallium, and the whole constitutes one branched gametophyte. In
the beginning of this experiment one sporophyte and also by acci-
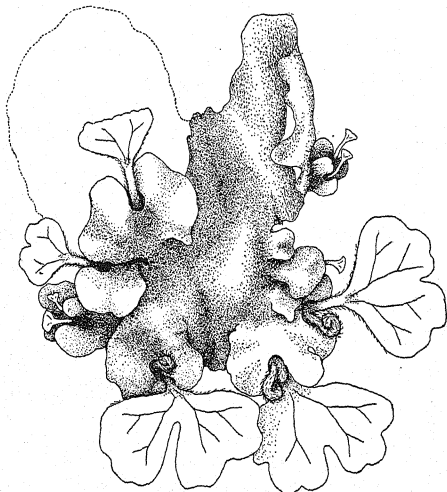


FIG. 2.—Enlarged drawing from photograph of prothallium of *Dryopteris mollis,*
showing eleven marginal proliferations, ten of which bear one rooted sporophyte
each; ×5.

dent the left wing of the gametophyte were cut away. The ampu-
tated wing is indicated by the dotted line.

Fig. 3 illustrates another interesting result in *Dryopteris mollis*.
Before proliferations began to develop on this prothallium, two
sporophytes were successively amputated at different times. In the
process of amputation the two lobes or wings of the prothallium were
slightly injured. They stood up almost at right angles to the midrib,

and soon withered, doubtless as a result of injury in handling. Before the outline drawing of this figure was made, the injured lobes were carefully cut away so that the proliferations might plainly be seen. These proliferations, which were mostly heart-shaped, numbered seventeen. They sprang from both sides and from the ends of the midrib, as shown in the figure.

Of these seventeen proliferations, eleven had produced a rooted sporophyte at the time of harvesting the specimen for critical observation. These lobes are all too small to produce such vigorous sporophytes unless attached to a parent plant. Each lobe or proliferation, whether heart-shaped or not, bore both antheridia and archegonia.

FIG. 3.—Diagrammatic drawing of prothallium of *Dryopteris mollis*, from which the first two sporophytes were amputated; subsequently the 13 or 14 proliferations developed from the sides of the midrib on the under side; eleven of these had borne as many sporophytes, making 13 in all; ✕5.

In cultures of *Dryopteris mollis* extending over two years, in which the plants were grown upon earth under the most varied conditions, not a suggestion of real apogamy was ever observed. That we are dealing with a single, although much branched gametophyte there can be no doubt, inasmuch as the proliferations or branches form one continuous tissue complex. The prothallia of figs. 2 and 3 are truly as individual as two maple trees.

Few plants outside the liverworts lend themselves more readily to experimentation than certain fern prothallia, especially when phenomena of regeneration are sought. Any part of the prothallium, if carefully severed at the proper time, and suitably cultivated, will regenerate a new individual.

The writer has not carried experimentation in the production of plural sporophytes on a single gametophyte further than the cases cited in the foregoing, for other phenomena came under observation in the series of experiments which seemed to offer interesting and important results. One of these was the behavior of the pro

thallia under prolonged cultivation, in which the plants were not
permitted to bear sporophytes. A brief preliminary report of meth-
ods and results was made before the Botanical Society of America
at the Cincinnati meeting of 1923. The cultures continue to reveal
such interesting and definite results, that publication of a more
complete report will be deferred to a later date.

### Summary

1. Under experimental conditions individual prothallia of *Dryop-
teris mollis* (Jaq.) Hieron., *Matteuccia nodulosa* (Michx.) Fernald,
together with certain other polypodiaceous species, and *Osmunda
Claytoniana* L. may be made to produce from two to many sporo-
phytes.

2. If the prothallia are permitted to attain a size of 1–1.5 cm.
in width across the wings before fecundation is effected, as high as
five independent and self-nourishing sporophytes may be developed
before the weakening or partial exhaustion of the gametophytes.

3. If the sporophytes are carefully amputated as soon as they
become plainly visible, a succession of sporophytes can be pro-
duced on an individual prothallium.

4. If the massive prothallia develop proliferations as a result of
the experimental cultivation, from ten to fifteen or more sporo-
phytes may be produced.

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BLOOMINGTON, IND.

# BRIEFER ARTICLES

## AN ABNORMAL ARCHEGONIUM OF FUNARIA HYGROMETRICA[1]

### (WITH ONE FIGURE)

In 1908 the writer[2] called attention to an abnormal archegonium
found in *Porella*, and referred to the literature bearing on somewhat
similar cases in other plants. The archegonium shown in
fig. 1 was found in *Funaria hygrometrica* which had been
grown in a greenhouse. The spores had been sown on
sterilized soil, and they produced in the usual way a great
number of plants which were densely crowded together.
It will be seen that the archegonium differs from the usual
type in having two egg cells, and, in addition to the usual
single chain of canal cells, part of a second row of two canal
cells and a ventral canal cell. The two egg cells are rather
large. The archegonium itself was somewhat larger than
the others observed. The figure was drawn with a camera
lucida after having been cleared in glycerine, as in my
drawing of *Porella*.

The position of the egg cells, ventral canal .cells, and
canal cells were placed side by side as shown in my figure
of the archegonium of *Funaria hygrometrica*. These cells
are placed in a row above one another in alternating
positions, as shown by COKER[3] for *Mnium* and by BLISS[4]
for *Polytrichum juniperum.*—F. M. ANDREWS, *Indiana
University, Bloomington, Ind.*
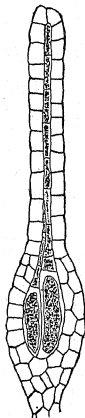
[1] Since the preparation of this article, a similar case has been found by EMIG
(Twin eggs in *Bryum caespiticium*. The Bryologist 1924: p. 94).

[2] ANDREWS, F. M., An abnormal *Porella platyphylla*. BOT. GAZ. 45:340. 1908.

[3] COKER, W. C., On the occurrence of two egg cells in the archegonium of *Mnium*.
BOT. GAZ. 35:136. 1903.

[4] BLISS, MARY C., On the occurrence of two venters in the archegonium of *Poly-
trichum juniperum*. BOT. GAZ. 36:141. 1903.

## FIFTIETH ANNIVERSARY ISSUE

The first number of the BOTANICAL GAZETTE, then known as the
*Botanical Bulletin*, was issued at Hanover, Indiana, in November, 1875.
With this issue, therefore, the Gazette reaches the golden anniversary of
its founding. It is an appropriate time to look back over the half century
of progress made since the Gazette began its career of service to botanical
science.

During the early years of publication, four to eight or twelve pages a
month sufficed to carry the material sent in for publication, and the
editor repeatedly urged botanists to send in notes of interest and value.
The notes were usually very brief, a paragraph or two, seldom a page in
length, and were mainly taxonomic, and geographic, interesting observa-
tions on plant distribution, unusual locations, local lists of species, or
comments on recent discoveries. Botany then was still dominated by the
naturalistic phases of the subject.

Publication in those days was relatively inexpensive. Although the
subscription price of the BOTANICAL GAZETTE in the beginning was only
$1.00 per year, and the subscription list about 100, the deficits were not
large, and after the first two years the Gazette paid its own way, even
without advertising matter, which was not admitted until the beginning
of the fourth volume, in 1879.

The BOTANICAL GAZETTE has made a wide appeal to botanists from
the very beginning. During the first ten or fifteen years after its founding,
it carried the names of a large array of noted botanists appended to the
published notes. ASA GRAY, whose interest was warm and personal from
the start, contributed frequently to its pages, and ENGELMANN, BESSEY,
VASEY, EATON, BEAL,`CHAPMAN, PECK, HOOKER, SARGENT, RUSBY,
BARNES, and many others made it their medium of communication with
their fellow botanists.

The growth of the BOTANICAL GAZETTE during the last forty years
reflects the enormous advances made by the science of botany during
this period. Morphology led the advance out of the naturalistic period,
with great contributions to our knowledge of the phylogeny of the great
orders of plants. A little later plant physiology and ecology shared in the
rapid development of our knowledge of plant life, and the Gazette has
carried in its pages many of the most important discoveries made during
the last quarter of a century.

Although the BOTANICAL GAZETTE began its career at Hanover,
Indiana, it has gone with its editor from place to place in his career as
investigator and teacher. From Hanover it went to Crawfordsville in

1879, to Bloomington in 1891, to Lake Forest in 1893, and finally to
Chicago in 1896. The printing has been done by the University of Chi-
cago Press for almost thirty years, and a very high standard of excellence
in form and workmanship has been established.

As the Gazette begins its second half century of service to botanical
science, the progress made during its career thus far gives us hope and
courage for the tasks of today and tomorrow. Although Professor JOHN
M. COULTER, founder and able editor of the Gazette ever since its found-
ing, is retiring from active service at the University of Chicago, he retains
the editorship of the journal, and the BOTANICAL GAZETTE looks forward
to an ever increasing service to botanical science, and through it, to
human welfare.—C. A. SHULL, *University of Chicago.*

# CURRENT LITERATURE

## BOOK REVIEWS

### Biology of the flowering plants

A book of more than usual interest and value to the general student of botany has been written by SKENE,[1] who bases his discussion of the higher plants on the firm foundation of experimental physiology. He has summarized the results of a large amount of the modern research upon plant life and plant behavior, and presents a wealth of detailed information, but does it so skilfully that the general picture of plant life is not distorted or obscured by the mass of details. It seems to the reviewer to be a very well balanced discussion, and is written in a style that holds the interest closely. It will be especially helpful to students who desire to keep a broad grasp of the facts while specializing in some particular field of botany.

There are only six chapters, but each covers a very broad field in the life of the plant. The first deals with the fundamental soil relationships, root systems, and the absorption of water and salts. This is followed by a long chapter on assimilation of carbon, and transpiration, which repays careful reading. Chapter iii considers special modes of nutrition, parasitic, saprophytic, mycotrophic, and symbiotic nutrition, and insectivorous plants. The last three chapters discuss mechanical problems and protection; reproduction and dispersal; and development.

The bibliography at the close of the work contains about 600 citations, and one finds among them most of the outstanding contributions in recent years. The American literature has been used freely, and good judgment is shown in the selection and presentation of the material. The fair minded critical spirit of the author is very commendable. All students who desire a well rounded view of the plant as a living organism should have it upon the convenient book shelf.—C. A. SHULL.

### Oecology of plants

The appearance of a second English edition of WARMING'S book on plant ecology[2] will indicate to a certain extent the importance of his contribution to this branch of science, and its growth since the first edition appeared in 1909. It is gratifying to recall that Professor WARMING lived to see the development of strong ecological schools in England and America, as well as in his native

[1] SKENE, MACGREGOR, The biology of the flowering plants. 8vo. xii+523. New York: Macmillan Co. 1924.

[2] WARMING, EUG., Oecology of plants. An introduction to the study of plant communities. 2d ed. Eng. transl. by GROOM and BALFOUR. 8vo. pp. xi+422. London: Oxford Univ. Press. 1925. $5.00.

Denmark, resulting from the stimulating influence of his "Plantesamfund" that appeared thirty years ago. This reprinting of the English edition of his great work is a fitting tribute to his memory.—Geo. D. Fuller.

## MINOR NOTICES

A simple guide to trees.—Curtis[3] has prepared a neat little book on trees, primarily for the use of boy scouts and campfire girls, but which will be useful for all who wish to readily identify our common trees without the usual technicalities and difficulties of the ordinary manuals. There is an illustration for almost every species presented, and the keys to genera and species are simplicity itself. The author has certainly succeeded in what he undertook to do.— H. C. Cowles.

## NOTES FOR STUDENTS

Tertiary plants from western states.—A series of papers has recently been published by Chaney, dealing with the distribution of certain Tertiary floras from western United States, and with their relation to groups of living plants now found in the same general regions. Chaney's investigations are not only interesting from the points of view of floral distribution and geological history, but he also draws important conclusions upon the ecological features as shown by these Tertiary floras.

The first paper[4] deals with the similarity between the Bridge Creek flora found in the Tertiary of the John Day Basin and other parts of Oregon, and the living redwood forest of the Coast Ranges of California. He discusses the principles governing the determination of fossil plants, and his own method in particular. He calls it the "ecological method," and the procedure is as follows. He first compares the fossil flora with a list of eligible genera at present found in the region where the fossils were deposited. When possibilities for matching these have been exhausted, the genera of the surrounding regions are considered, and then the genera of the continent. This method differs considerably from that pursued by the paleobotanists of the older schools like Heer, Ettings-hausen, Lesquereux, who did not hesitate to connect the fossil plants of their localities with the species and genera found in tropical and distant regions. This fact may account for the great mixtures of floral elements which those men claimed to have observed in the Tertiary.

After a careful study of the composition of the Bridge Creek flora, Chaney discusses the climatic and topographic indications, and their geologic correlation. He concludes that the Bridge Creek flora is found in Oregon in beds of probable Oligocene age, and that a similar group of plant fossils is scattered widely in the Tertiary of the Northern Hemisphere. It has a most conspicuous

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[3] Curtis, C. C., A guide to the trees. 8vo. pp. 208. figs. 207. New York: Greenberg, Publisher, Inc. 1925.

[4] Chaney, R. W., A comparative study of the Bridge Creek flora and the modern redwood forest. Carnegie Inst. Washington, Publ. no. 349. pp. 22. 1925.

element formed by a group of species whose modern relatives are now represent-
ed in the redwood forest flora, and because of this relation the physical condi-
tions under which the Bridge Creek forest lived were probably similar to that
of the present day redwood forests of the Coast Ranges of California. The
climate appears to have been temperate and moist. The shales containing the
Bridge Creek fossils are made up largely of volcanic material which was blown
and washed into basins of deposition in forested valleys.

The second paper[5] deals with the Mascall flora located on the Van Horn
ranch near Dayville, Oregon, on the east fork of the John Day River. The same
method is applied here as in the paper previously discussed. The Mascall flora
is common in the Great Basin portions of Washington, Oregon, Idaho, Nevada,
and California, but occurs also in west-central California, where its composition
is less typical than in the Great Basin. It had been described by KNOWLTON[6]
more than twenty years ago, and fine collections of the Mascall flora are found
in the New York Botanical Garden, the American Museum of Natural History,
and Princeton University. CHANEY concludes that the Mascall flora belonged
to the Middle or Upper Miocene, and that it represents a climax forest formation
which would indicate a lower relief and a climatic condition similar to the one
at present in this same region.

The third paper[7] deals with a new fossil leaf species from the John Day
series of Oregon, and with a description of a fruit species from the Fork Rivers
formation of South Dakota. The author offers the following explanation for
differences in character of the Celtis record in the Bridge Creek shales and in
the White River clays. The Bridge Creek shales were laid down in a moist
region, as indicated by the character of the flora as a whole, and the hackberry
trees appear to have occupied the forest borders and the slopes at some distance
from the water bodies. The White River clays were laid down in an arid or
semi-arid region, as indicated by the almost complete absence of fossil leaves,
and the hackberry trees occupied the immediate borders of the intermittent
water bodies. CHANEY suggests, on the basis of the fossil and living hackberries,
that climatic changes and the resultant vegetational development in South
Dakota and Oregon have been somewhat similar from the Tertiary up to the
present time, but that in Oregon they have been lagging behind the changes in
South Dakota.

CHANEY'S fourth paper[8] deals with Umbellularia, a genus of the laurel
family, which is now limited to western California and southwestern Oregon,

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[5] CHANEY, R. W., The Mascall flora, its distribution and climatic relation. Car-
negie Inst. Washington, Publ. no. 349. 23-48. 1925.

[6] KNOWLTON, F. H., U.S. Geol. Surv. Bull. 204. 1902.

[7] CHANEY, R. W., Notes on two fossil hackberries from the Tertiary of the Western
United States. Carnegie Inst. Washington, Publ. no. 349. 49-56. 1925.

[8] CHANEY, R. W., A record of the presence of Umbellularia in the Tertiary of the
western United States. Carnegie Inst. Washington, Publ. no. 349. 57-62. 1925.

reaching its greatest abundance and largest size in the former state. In connection with his studies of the John Day and other Tertiary floras in the west, the author has collected a considerable number of leaf specimens that appear to be referable to this genus. He has counted over twenty thousand fossil specimens at the Bridge Creek locality, and found that the recent *U. integrifolia* made up 8.82 per cent, which indicated to him that its representation in the Oligocene redwood forest was on the same order as that of the living species in the related forests of western California. This species, which resembles so closely the recent *U. integrifolia*, was named *Fraxinus integrifolia* by NEWBERRY in 1882. It is called by CHANEY *U. oregonensis*, since generically it is indistinguishable from the living California laurel *U. californica*.—A. C. NOÉ.

Mycorrhiza in the Ericaceae.—Continuing her researches on *Calluna vulgaris*, Miss RAYNER[9] is unable to confirm the results of CHRISTOPH,[10] who claimed that he had produced vigorous seedlings on sterilized soil with roots entirely free from any fungal infection. All seedlings from unsterilized seed seem liable to infection from fungi in the seed coats, in spite of CHRISTOPH's assumption that infection by the mycorrhizal fungus takes place only from soil.

A later contribution by RAYNER[11] shows that the endophyte has been isolated and identified as belonging to the genus *Phoma*, and that in association with the heather plants it is able to utilize atmospheric nitrogen. While seedlings of *Calluna vulgaris* seem incapable of growth unless infected soon after germination, the view is expressed that plants would probably grow quite well without mycorrhiza, could seedlings be raised free from infection. In nature the formation of mycorrhiza in all roots is the rule. The development of the endophyte in mycorrhizal cells is markedly inhibited by the low soil temperatures of early spring, and also under experimental culture conditions by sterilized soil and sand cultures. A digestion of the mycelium in the mycorrhizal cells is shown to continue during the growing season, and there seems to be an exchange of nutritive material between the fungus and the vascular plant, with the "balance of profit" on the side of the latter.—GEO. D. FULLER.

Drying timber.—Up to rather recent times, as PATTON[12] points out, air seasoned timber was used in all the best woodwork, but now this method does not supply enough lumber, and some at least must be kiln dried. The research reported on in this paper had for its object the study of the factors involved in

⁹ RAYNER, M. CHEVELEY, Mycorrhiza in the Ericaceae. Trans. Brit. Mycol. Soc. 8:61–66. 1922.

¹⁰ CHRISTOPH, H., Untersuchungen über die mykrophyten Verhältnisse der Ericales und die Keimung von Pirolaceae. Beih. Bot. Centralbl. 38:115. 1921.

¹¹ RAYNER, M. CHEVELEY, The nutrition of mycorrhiza plants: *Calluna vulgaris*. Brit. Jour. Exper. Biol. 2:265–292. 1925.

¹² PATTON, R. T., On the drying of timber. Proc. Roy. Soc. Victoria (N.S. Pt. I) 35:63–85. 1922.

the drying of timber, and their application to the commercial seasoning of timber. The six factors studied were moisture content, diffusion of moisture, evaporating surface, thickness, humidity, and temperature. Most of the trees studied had a higher moisture content in the sapwood than in the heartwood. The diffusion constant for a number of trees was determined. The oak, for example, has a high moisture content and a low power of diffusion; it is therefore difficult to season. The pine, on the other hand, has a low moisture content and a high power of diffusion; it therefore dries rapidly. Water was lost most rapidly from the transverse surface, that is, in the direction in which the water moves in the tree, and least rapidly from the radial surface; while the loss from the tangential surface was but little more than from the radial. The rate of water loss was independent of the thickness of the block up to a certain point, then, as drying proceeded, the thinner block lost water less rapidly. The influence of different combinations of temperature and moisture were studied. Studies of this kind should result in improved methods of seasoning lumber, especially where kiln drying is the method used.—S. V. EATON.

Virgin forests of Ohio.—In an interesting investigation of the original vegetation of Ohio, SEARS[13] has used as sources of information the records and field notes of the first land surveys of the State, dating back to 1786, county histories, and the journals of travelers. The most abundant trees seem to have been oak, beech, and ash, representing respectively forest associations of (1) *Quercus* and *Carya*; (2) *Fagus grandifolia* and *Acer saccharum* with a sprinkling of *Fraxinus americana* and *Quercus rubra*; and (3) *Fraxinus americana, F. nigra, Ulmus americana*, and *Acer saccharinum*. The distribution of these associations within the glaciated area seems primarily correlated with the system of morainal deposits. Unmixed beech appears to have been limited to the glaciated region, and ash to have been best developed within the Erie plain. Maps present the results of these studies in graphic form.—GEO. D. FULLER.

Classification.—CAMPBELL[14] has made some suggestions in reference to a classification of the plant kingdom "more in harmony with our present knowledge" than "the very antiquated system still in vogue." His suggestions are well worth considering. They involve four primary divisions: Protophyta (including the troublesome Myxomycetes, Flagellates, and Schizophytes), Algae, Fungi, and Embryophyta (Bryophytes, Pteridophytes, and Spermatophytes). It is interesting to note that the four classes of Spermatophytes dispose of the old categories of gymnosperms and angiosperms, being Cycadophyta, Coniferae, Gnetales, and Anthophyta (angiosperms).—J. M. C.

[13] SEARS, P. B., The natural vegetation of Ohio. I. A map of the virgin forests. Ohio Jour. Sci. 25:139-149. 1925.

[14] CAMPBELL, D. H., Some suggestions on classification. Science 61:403-405. 1925.

# THE

# BOTANICAL GAZETTE

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## PROFILES OF PEATLANDS WITHIN LIMITS OF EXTINCT GLACIAL LAKES AGASSIZ AND WISCONSIN

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(WITH THREE FIGURES)

The present paper is a further attempt to show the connection between profile sections of peatlands and the environmental conditions which determined the formation of peat deposits in this country. The chief controlling field condition, which is here emphasized, is of exceptional importance in so far as it has a bearing on the selection and relative value of peatlands for different uses.

For Ohio peat deposits, a former paper (7) outlined the greater stratigraphic complexity of certain peatlands found within the older morainal belts. Although not all peat deposits necessarily date back to early postglacial times, the profile sections varied in more or less direct relation to the conditions which caused the successive positions of the receding ice sheet.

In a more recent article (11), it was shown that in the New England states the individual profiles of widely separated peat areas could be correlated. They confirmed the little that is known about the recessional moraines in that region. The study assigned to these deposits an approximate date, which, although not as yet expressed as so many years before the present, could nevertheless be referred to a certain time estimate in which the formation of the peatlands began in the respective localities. Among other things, the fact that

stratigraphic sections of different peatlands do not follow the usual
order of the successional trend observed in the zonation of surface
vegetation, was cited as an indication of changes in the external
conditions, chiefly the amount of water from precipitation, which
attended the peat formation.

The additional material upon which the present paper is based,
was collected several years ago in Minnesota (1919) and Wisconsin
(1922).[1] The purpose of the field study was to learn how far and in
what ways the order of peat layers in western states depends on
differences in external conditions, and to check up correlations con-
nected with records from flooded valleys, ponded lakes, and plains
which resulted at the closing stage when the ice sheet withdrew in
the western region of the Great Lakes. The conditions to which the
glacial lakes Agassiz and Wisconsin owed their existence have
received a large share of attention from geologists. It seemed de-
sirable, therefore, to study briefly the pedomorphic structure of the
peat deposits which overlie these extinct lake beds. In this connec-
tion an attempt was made to evaluate the several field conditions
that are usually cited as controlling the formation of peatland.

In regard to the method of investigating peatland, it is important
to emphasize that accurate field information depends largely on the
botanical identification of the plant remains which form more or less
distinct and separate layers of peat. DAVIS (12), HUELS (14),
SOPER (25, 26), and others have mentioned the botanical char-
acter of peat as a means of studying and classifying respectively
the Minnesota and Wisconsin peat resources. The connections, how-
ever, which SOPER and OSBON, and preceding them DAVIS supposed
they had made between "turfy, fibrous, earthy, and pitchy peat"
and the botanical composition of the several layers of plant remains
cannot be right, because these descriptions are not characteristic.
Moreover, they have not made the botanical constituents a basis
either for establishing stratigraphic differences, for identifying and
coordinating layers of peat in widely separated localities of a region,
or for working out the records made by the migrating vegetation,

and the history of past environmental factors. On the contrary, these writers and others have attached much scientific interest to the general laws relating to the sequence of surface vegetation on peat deposits. The ecology of peatlands, indeed, may be regarded as a subject of its own for a correct understanding of the relationship of plants to their environment and the conditions under which vegetation of different types can grow. But peat deposits have been subject also to changes, in degree depending upon the time element involved in their formation. The limitations of a system of classifying peatland based on the sequence of surface vegetation have been briefly considered in a previous paper (6).

A detailed account of the writer's field practice has been given elsewhere (9), together with photographic illustrations of different kinds of peat and the chemical analyses of certain of their organic substances (10). Charts of profile sections, to show the widely varying differences in peatland, were included in several of the papers. In these pedomorphic studies of correlating series of layers of peat at one locality with the layers of plant remains in another locality, the starting point has been made for new peat investigations, which will be apparent to any one examining the data. Clearer conceptions are now possible concerning type profiles and the physical and chemical composition of the different layers of peat. A better understanding may be obtained as to the nature of the transformation of plant remains from peat to muck and humus, and the products brought about by the activity of microorganisms. Profile records will bring out the conditions under which drainage, cultivation, fertilizing, and systems of farming should take place. Agricultural as well as industrial problems in peatland utilization will see their practical solution when a thorough line of observation is made in each important peat area. Through the measurement and coordination of profile sections, the experimental and economic correspondences can be finally made for regional groups of peatland selected on a stratigraphic basis. A chief disadvantage has been the lack of adequate field data. It is hoped that other investigators may become interested, so as to secure and study in detail a greater number of profile sections, particularly in areas which are physiographically uniform.

I. Profiles of peat deposits in area of glacial Lake Agassiz

Glacial Lake Agassiz covered much of the Red River basin and the northwestern portion of Minnesota (fig. 1). It extended westward into the prairie region of North Dakota, and northward far into Manitoba and Ontario, Canada. UPHAM's monograph (27) contains many important data on this former lake. Nearly its entire
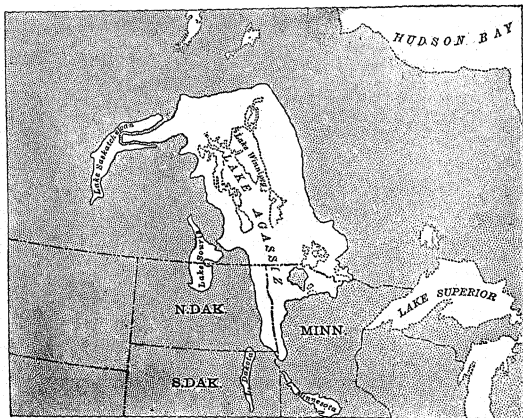


Fig. 1.—Map of extinct Lake Agassiz, and other glacial lakes (UPHAM, U.S. Geol. Survey).

bed rests upon calcareous gray drift, which had been carried in previously by the Keewatin ice sheet.

The waters forming glacial Lake Agassiz were held back on the north in central Canada by glaciers, and for a long time they were ponded in front of the ice sheet. Preceding the development of this ancient lake there was a temporary older body of water. The ice that covered Ohio and the southeastern portion of this country had melted, while in the northwestern states glacial conditions appear to have been stationary through a long period. With the melt-

ing back of the northern ice border, however, the pre-Agassiz lake
grew from south to north as the ice sheet receded. The highest stage
of the waters in this largest of the many Pleistocene lakes was prob-
ably 500 feet above the present surface of Lake Superior (602 feet).
In much of this area the waters were relatively shallow, and hence
there were several islands in the lake. The level of the waters was
lowered from time to time, forming beaches of sand and gravel along
its shores. Several large deltas were formed contemporaneously
with the higher shore lines, and for some time the lake probably re-
ceived modified drift brought by streams from the receding glaciers.
Gradually the lake was drained to the south through the Minnesota
River (glacial river Warren at that time), partly by the cutting down
of the outlet and partly by an uplift of this region.

The shores of Lake Agassiz generally stand 5–10 feet, and occa-
sionally 15–20 feet above the lake washed till of the clayey and limey
plains. Later, it seems, the lake area was again encroached upon by
the ice sheet, since the shore lines are obliterated in places, except
along the eastern side. Finally there was a change of outlet from the
southern to the northern end. The waters escaped eastward into the
Lake Huron basin, and later to the much enlarged Gulf of St. Law-
rence. With the uncovering of Hudson Bay, the lake receded to its
present position in Manitoba, where Lake Winnipeg is one of the
remnants.

With the recession of the lake waters from the southern borders
in Minnesota, large areas of land were uncovered. The southern
and eastern borders of the lake were the first to become dry, and to
be converted into marshes and forests by the incoming vegetation.
Gradually these and the intervening isolated ponds and small lakes
became the site of the extensive, flat peatland of today. UPHAM
estimates that the recession of the ice sheet causing Lake Agassiz
may have been from seven to ten thousand years ago, and that
the duration of this great lake was probably not more than one
thousand years. On the basis of this surprisingly short estimate for
the duration of Lake Agassiz, therefore, the formation and accumula-
tion of layers of peat have occupied not more than six to eight thou-
sand years. In the course of that time to the present, several layers
of peat have been formed in this region. The levels of the changing

surface vegetation and of the fluctuating ground water table were slowly raised, and the inequalities of the underlying topography, as well as the variety of mineral soils, disappeared under one of the largest peatland areas in Minnesota. To the pioneer settlers, nearly the entire portion of extinct Lake Agassiz in northern Minnesota was an unbroken expanse of "muskeg" swamp, in part timbered with spruce and tamarack, in part with open heath bogs and sedge marshes.

In the following paragraphs a description is given of some of the profile sections of the great muskegs which now cover a considerable portion of the ancient lake bed in Beltrami, Koochiching, and Roseau counties. The location and acreage of this peatland area are shown on LEVERETT'S map of the surface formations (15, 16), and on the topographic and drainage map which accompanies House Document 27 of the Sixty-First United States Congress, first session. More recently the engineers in charge of state drainage projects have surveyed and sounded the depth of much of this peat area. Part of their data and maps have been included in SOPER'S report on the peat deposits of Minnesota (25). Of the writer's data and field-work, only as much is given here as is necessary to show the connections among peat profiles from widely separated localities of the same lake bed.

1. SOUNDINGS IN T. 153 N., R. 26 W., S. 16, ABOUT 50 FEET WEST OF THE PUBLIC ROAD.—The vegetation consists of a good growth of tamarack, cedar, and a few spruce, with heath shrubs such as *Ledum* in sphagnum mosses. Burned and cleared portions show *Potentilla fruticosa*. In the open state ditch nearby a horizon of lime accumulation is beginning to appear; it is readily recognizable by the lighter color and the effervescence upon application of acid. At this locality the depth to the underlying calcareous sandy clay varies between 5 and 8 feet. The several layers of peat are easily observed for a distance of several miles. Their order and thickness in the profile section of this vicinity are as follows, enumerated from the top downward.

Sphagnum peat: 6–8 inches; fibrous, relatively dry and light brown in color.

Woody peat: 1 foot in average thickness; dark brown, rather compact,

granular, with roots and stumps of conifers and woody fragments of various
sizes; this grades into
    Sedge peat: 3.5 feet; brown, finely fibrous, partly disintegrated, in places
somewhat coarse and matted; sandy at lower level, with roots in gray sandy
to gravelly clay.

    2. TEST-BORINGS IN T. 154 N., R. 25 W., S. 21, ABOUT 200 FEET
EAST OF MINNESOTA AND INTERNATIONAL RAILROAD.—This is the
continuation of the tamarack-spruce-cedar muskeg already de-
scribed. The layers in the profile section have the following sequence
and thickness:

    Sphagnum peat: 7–9 inches; light grayish brown, largely fibrous.
    Woody peat: 12–14 inches; brown to dark reddish brown; consists largely
of the woody fragments and granular remains of coniferous trees, with cones
and needles; grading into
    Sedge peat: 4–5 feet; brown, largely fibrous.
    Woody peat: 1.5–2 feet; dark to very dark brown, in part poorly decom-
posed showing stumps and woody fragments of conifers with fibrous débris from
roots; the underlying mineral is brownish gray, clayey sand over a bluish gray
clay.

    This profile section shows very good agreement with different
test-borings in such portions of the same muskeg as are situated
near Nakoda, Little Fork, and Wisner.

    3. CONTINUATION OF MUSKEG SWAMP; SOUNDINGS IN T. 154 N.,
AND R. 25 W., SOUTHWEST OF BIG FALLS, MINNESOTA.—The test-
borings show essentially the same profile, but the thickness of the
layer of sedge peat is greater and includes a pocket of standing water
between an upper and lower mat of sedge peat. The surface vege-
tation consists in places of scrub tamarack and spruce, but varies
within relatively short distances. The depth of peat accumulation,
however, bears no relationship either to the size or to the abundance
of the conifers present. This supposition is strongly supported by
the photographs which accompany SOPER's report (25). They show
that in some of the largest and deepest peat areas tamarack and
spruce are present in about equal numbers, without any tendency
toward segregation or dwarfing. It must be assumed that some
other factor or factors are decisive in determining the quality of
timber produced. Field observations show that the volume of wood
produced varies periodically. The pathologic condition which

lowers the quality and height of the timber appears to result from
a reducing action and a slow rate of decomposition of the more resist-
ant sedge or reed peat in the rooting horizon utilized by the forest
trees. A slow rate of tree growth is to be expected when the ratio
between the carbon and nitrogen content is in an unfavorable condi-
tion for decomposition, or when the water content is too high for any
period of time, irrespective of the degree of decomposition and the
type of the peat material which is occupied by the roots of the
trees (10). The evidence from buried forests in peatland profiles is of
a similar nature over a wide range of the country.

4. SOUNDINGS IN T. 154 N., R. 26 W., S. 11.—This is a part of
the great muskeg swamp with spruce and tamarack covering several
hundred square miles in Beltrami and Koochiching counties. Test-
holes along a newly constructed road and observations in an open
ditch show the order and thickness of layers as follows:

Sphagnum peat: 9–10 inches; gray brown, fibrous, moist.

Woody peat: 1 foot average thickness; chiefly the remains of a spruce and
tamarack forest with charred woody fragments at lower level; this grades into

Sedge peat: 6–8 inches; brown, firm, partly fibrous.

Hypnum peat: 5–7 inches; yellow brown, poorly decomposed, with an
admixture of fibrous material from sedges at upper level; fairly compact.

Woody peat: 1.5–2 feet; dark brown to very dark reddish brown, derived
largely from tamarack and spruce; contains material from ferns, cones, and
needles; layer rests on a brownish sandy clay which grades into light colored
gray, sandy clay.

SOPER does not give profile sections in his bulletin on the peat
deposits of Minnesota; consequently, the actual sequence and thick-
ness of the individual layers of peat have not been worked out by
him. So far as there is material for identification, however, SOPER's
description of the peatland in Beltrami and Koochiching counties
shows certain agreements with the botanical characterization of the
layers of peat just enumerated, and the correspondences between
the different peat profiles are rather good. The agreement is quite
striking regarding the basal layer of woody material which SOPER
has recorded between points at distances of many miles. According-
ly, this shows a very wide spread of an ancient forest, with spruce
and tamarack as its most typical constituent. This forest appears
to have taken possession of the lake bed wherever local conditions

were not extreme. Climatologically this area must have been rela-
tively uniform, for the forest vegetation undoubtedly indicates that
the ground water table was low. The trees were growing under very
favorable conditions for a considerable period of time, since the layer
of woody peat is thick at all localities measured. The ice front must
have been fairly distant.

Obstructions in drainage and a rise in water level evidently
played a prominent rôle in the formation of the succeeding layers of
*Hypnum* and sedge peat. These layers indicate rather strong dis-
turbances. The presence of *Hypnum* mosses as a layer overlying
woody peat is difficult to understand, and the circumstances under
which the organic material was deposited are not clear. In SOPER'S
account this section of the profile series cannot be identified, since his
descriptions are not characteristic for corresponding situations. In
this vicinity the *Hypnum* layer is relatively thin, and appears to
have accumulated in shallow water. Farther north, near Algoma
in Roseau County, the layer is present in considerable thickness, and
contains an admixture of somewhat coarse plant remains from spe-
cies of *Equisetum* and sedges. It is not unlikely that *Hypnum* peat
represents conditions of growth in cold glacial waters which came
from an ice sheet readvancing to a point some distance north of this
locality. When the ice again withdrew, and outlets for glacial waters
were uncovered, the stages to sedge peat and finally to the upper-
most level of woody forest peat may correspond with the changes in
water level and the shifting of the climatic conditions accompanying
the ice recession into Canada.

The profiles enumerated correspond with those which were out-
lined for peat deposits at Chassel, Michigan, occupying the marginal
portions of the glacial Lake Algonquin (8).

II. **Profiles of peat deposits within area of glacial
Lake Wisconsin**

This former body of water was in the Driftless Area between the
Wisconsin and Black Rivers. It probably covered at least 1825
square miles. The extent and form of glacial Lake Wisconsin and
the positions of some of its glacial streams are shown in fig. 2, from
MARTIN (18). The lake was held in by the margin of the ice sheet

to the east of the Driftless Area. The waters rose to a height of 960–1000 feet above sea level, being about 70–150 feet deep. Clay, sand, and erratic bowlders from floating icebergs constitute the chief deposits of this lake; they form a relatively impervious bottom over the porous Cambrian sandstone underlying this area.

Glacial Lake Wisconsin is believed to have been relatively short lived; it produced few well defined shore lines. At present these shore lines are not horizontal, but tilted by an uplift. They rise toward the north, and increase in altitude from 960 feet near Baraboo and Kilbourn to almost 1000 feet to the north about 55 miles, near City Point. When the ice had melted back from the valleys near Baraboo, the waters of glacial Lake Wisconsin cut through the terminal moraines to the southeast until the lake was drained out. The waters may have escaped to the west through the Black River outlet, however, since deposits of this lake extend out into the plain west of the Black River divide. It is not yet known at what time glacial Lake Wisconsin ceased to exist. It appears to have been coexistent with glacial Lake Agassiz.

After the lake was drained, rather large streams seem to have been at work, spreading outwash gravels and other material over some areas within the lake basin. Subsequently the lake deposits were subjected to wind action. In many places dunes and loess-like material were formed. In the northern and northwestern part of the basin the lake deposit is white quartz sand, owing to the large area of crystalline rocks in the north. This lake bottom sand, underlain in turn by impervious clay, is now the mineral subsoil of large areas of peat, and may be well seen along the extensive drainage ditches of Wood, Juneau, and Jackson counties. The finer detritus was carried farther off shore, and spread over the lake bottom as calcareous clay. In the southern part of the lake basin the soils are reddish silts and sandy clays.

Today glacial Lake Wisconsin represents the largest peatland area in the state of Wisconsin. It is known generally as the Great Swamp of Central Wisconsin (fig. 3). Maps showing acreage and location are given by MARTIN (18). Its surface vegetation included numerous open sedge and reed marshes with no timber, as well as

areas forested with tamarack, spruce, and occasionally white pine.
The accumulation of peat varies in depth, and covers a variety of
topographic features and mineral soils over an area of nearly
300,000 acres. At present parts of this extensive deposit are used
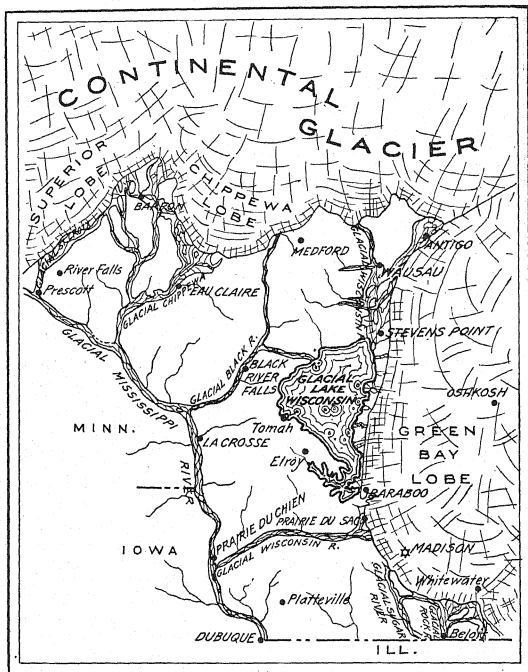


FIG. 2.—Driftless area at Wisconsin stage of glaciation (MARTIN, Wis. Geol. &
Nat. Hist. Survey).

for cranberry fields; in others sphagnum moss is gathered and shipped to florists. Relatively little of this area appears to be under cultivation in general farm crops.
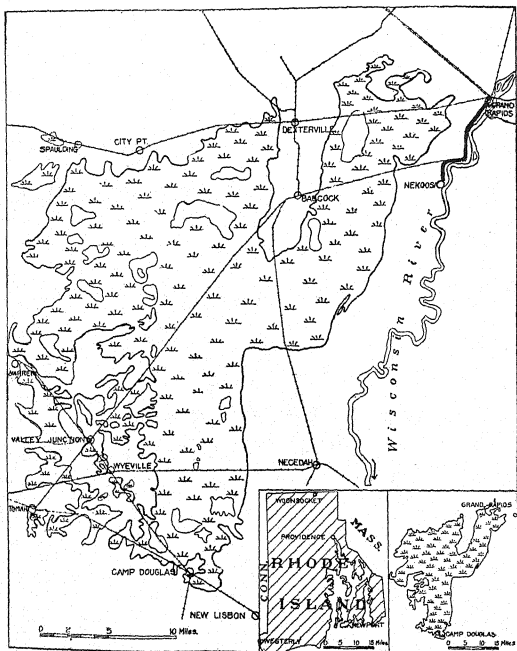


FIG. 3.—Great swamp of central Wisconsin (MARTIN, Wis. Geol. & Nat. Hist. Survey).

A few of the individual peatland profiles will be discussed, with comments on the cross-sections which occur in the same areas at widely separated localities. As in the profiles from the region of gla-

cial Lake Agassiz, all minor variations in plant remains and distur-
bances due to quite local conditions have been discarded, or merged
into the chief layers which showed characteristic botanical features
and average conditions.

1. WARRENS, WISCONSIN.—The profile section of this locality is
northeast of Warrens in T. 20 N., R. 1 E., in the southeast quarter of
Section 28. The area is under cultivation, and has been burned over
repeatedly. It is reported to have had in places a surface vegetation
which consisted of tamarack with heath shrubs, and a ground cover
of sphagnum mosses and other plants characteristic of a northern
conifer swamp. The profile, as shown by test-borings, which may
also be observed in the large open ditch draining this part of the peat-
land area, is as follows:

Sphagnum peat: burned off.
Woody peat: largely burned off; woody fragments from stumps and roots
at intervals.
Sedge peat: 5 feet in thickness; brown to dark brown in color, fibrous,
somewhat matted, and in part disintegrated.
Woody peat: 1 foot in average thickness; dark to very dark brown, largely
well decomposed, with roots and stumps of tamarack and spruce; underlying
mineral soil is gray siliceous sand.

In striking agreement with this profile are the cross-sections from
the "Leola Marsh" near Plainville, and from the "Buena Vista
Marsh" near Coddington. The latter contains a thin bed of gray
loess-like silt and a layer of *Hypnum* peat below the basal forest
peat. The profiles resemble closely the cross-section from peatland
at Lake Forest near Madison, and at Cottage Grove, Wisconsin.
Considering the different position of the localities in relation to the
glacial lake bed, the correspondence of the profiles is good and their
thicknesses agree well.

2. CITY POINT, WISCONSIN.—This peat area is an isolated por-
tion of the Great Swamp of Central Wisconsin, and is located west
of City Point in T. 22 N., R. 1 W., S. 26. The original vegetation in-
cluded tamarack and spruce with heath shrubs. At points about
600 feet south of the public road and the Green Bay and Western
Railway, test-holes show the following profile features:

Sphagnum peat: 8–10 inches; gray brown, fibrous.
Woody peat: 4–6 inches; dark reddish brown coarsely fragmented with

fibrous material from sedges into which coniferous trees and various shrubs
spread their roots at different ranges of depth.

Sedge peat: 7–8 feet in thickness; brown to dark brown; varied in texture
from finely fibrous to matted material, frequently with an admixture of coarse
plant remains from reeds; at lower level the material is more or less disintegrated
in place, with occasional root fibers in a brown colored sandy organic débris
which grades into gray, compact siliceous sand.

3. Similar profiles to that found near City Point, Wisconsin,
were observed in the continuation of this same great swamp be-
tween Tomah and Wyeville, in a white pine-tamarack swamp in
T. 20 N., R. 1 E., S. 20., and at a locality in Wood County referred to
as the Searles cranberry marsh. They show a rather good corre-
spondence with one another. At the Searles locality the profile sec-
tion was obtained in a large drainage ditch which was under con-
struction at the time of visiting this vicinity. The thickness and
botanical composition of this peatland area were exposed in the
sides of the ditch for a considerable distance. The surface vegetation
consisted of tamarack, spruce, heath shrubs, and a varying ground
cover, chiefly of sphagnum mosses. The underlying mineral soil is
sand.

The report of HUELS (14) on the peat resources of Wisconsin
does not contain information relative to the counties within the
limits of glacial Lake Wisconsin. HUELS includes from the survey
of 1903 the description of a deposit three miles northwest of Bab-
cock in Wood County. This record seems to indicate a profile similar
to those just mentioned.

III. Relative importance of conditions which controlled
formation of peatland in glacial lakes Agassiz
and Wisconsin

The question now arises as to the basic conditions which de-
termined the formation of peatland after the draining of the glacial
waters. This problem is particularly important, whether relating to
forest trees, soil microorganisms, or farm crops.

The processes of peat formation are probably essentially the same
at the present moment as in the period immediately following the
recession of the ice sheet, but not so the general external conditions
under which the different layers of peat originated. It is generally

stated that the most important factors in the development of peat deposits are topography, soils, geology, and climate. These conditions are not infrequently cited as determining the main possibilities, as well as the main limitations of peatland agriculture. It is not purposed to enter into an extended discussion of the physical basis of peatland formation in the United States. The object here is to discuss briefly the larger environmental relations recorded in the profile of the peatlands within the ancient lake beds, with such references to the literature as point the direction of more special subjects of investigation.

1. The influence of the topography of any underlying land surface is usually cited as the most important external condition in the origin of peat deposits, as well as in their distribution and classification. This attitude is frequently expressed in the statement that, no matter how favorable the climate or the soils may be, peat cannot accumulate unless the topography is such that lakes, ponds, or other poorly drained depressions prevail.

It is customary to speak of any large portion of land as a topographic unit when it represents broad similarities among certain features, such as have been expressed, for example, for the glacial lakes Agassiz and Wisconsin. Nevertheless, the idea of a topographic unit cannot be applied to the beds of the two extinct lakes in an absolutely rigid manner. The description of these large basins shows that marked dissimilarities prevailed in geologic structure, physiographic processes, and time of development in the postglacial cycle. Similarly, topographic features, such as slopes, undulating surfaces, barriers, and depressions due to old stream channels and erosion varied from point to point in the two lake beds; yet examination of the peat profile sections shows conclusively that nearly everywhere plant remains were deposited and transformed into layers of peat in a relatively dry bottom of a flat, but not level lake bed. The great horizontal extent of the basal forest peat, the high degree of parallelism in the profile sections from widely separated localities, and the recognition that these extensive peat areas originated after the draining of the glacial waters, all these facts indicate that peat deposits are far from showing an exclusive relation to topographic conditions. The peat areas under discussion were not con-

fined to any one feature of topography, and they are not the effect
of varying subsidence. To what extent the original drainage chan-
nels have been cut deeper as the land rose cannot be said; at present
the peatlands do not disclose the character of the underlying land
surface. The topographic variations have all been smoothed over
and covered with several distinct layers of peat differing in origin and
composition. Neither the horizontal areal extent, nor the vertical
level which marks the present stratigraphic limit of peatland forma-
tion seems to have any direct bearing on topographic features.

2. The effect of underlying mineral soils upon the formation and
distribution of peat deposits has been the source of much controversy
in the literature which deals with the dependence of peatland on
environmental conditions. SENDTNER (22) and LORENZ (17) were
probably among the first to state that the distribution of vegetation
on peat deposits depends upon the chemical composition of the
underlying soil and soil waters. The absence of lime was regarded
as the characteristic feature, but not acidity. WEBER (29) assumed
that lake basins are successively modified in content of mineral
matter, such as lime, when the depressions become filled with peat.
The term eutrophic is used by him for peat materials formed in water
rich in mineral nutrients; oligotrophic for types with water poor in
saline food constituents; while mesotrophic is applied to peat layers
in the intermediate stage. ZAILER and WILK (31), BERSCH (4),
BIRK (5), MINSSEN (20), and WARÉN (28) give a large number of
chemical analyses. Their results show the changes of the mineral
matter in different kinds of plants which form peat, and in the
corresponding peat material derived from them. These investigators
found that the ash content does not increase with the depth of a de-
posit, but varies with the character of the profile section and with
the contamination by extraneous silt, lime, or ferruginous com-
pounds. It is important to note at this point that certain layers of
peat, when in contact with water, lose practically all the alkaline
salts and phosphates entering into the composition of the vegeta-
tion from which they were derived (10). The elimination of oxygen
from the organic débris also is proportionally much greater than that
of the other elements, as the water table becomes elevated with the
formation of peat. The peat materials become increasingly unsatu-

rated (3), that is, they show reduction action and are relatively more
acid, but this acidity follows the accumulation of peat; it does not
cause the occurrence and distribution of peatlands. The acid reac-
tion of the surface horizon of a peat soil does not necessarily indi-
cate the varying intensities of the reaction of the underlying layers
of peat or of the mineral subsoil. Moreover, PAUL (21), SKENE (24),
MEVIUS (19), and others have not been able to find any very marked
correlation between the acidity of a peat soil and its cover of vegeta-
tion. It thus appears unlikely that acidity alone is producing the
observed differences in the stratification of peatland or in their
surface vegetation. It must be realized also that the microorganic
population of a peat layer is sensitive to the reducing action of the
medium, and that fluctuations in the oxygen content will presum-
ably produce a marked effect, both on the species and numbers of
such soil microorganisms, and on the rate of the decomposition of
peat.

From an inspection of the geologic maps of Minnesota and Wis-
consin, it will be seen that peat areas in these states are not confined
to any one geologic formation. Furthermore, the recent mapping
of the boundaries of the drift sheets by LEVERETT (15, 16), and of
the diversity of soils by the United States Bureau of Soils have
made available many data bearing upon this problem. These facts
show that the peat area, found in the bed of former Lake Agassiz,
rests in large part upon calcareous gray drift, while the Lake Wis-
consin peatland, in the Driftless Area of that state, is not confined
to any one drift cover, but occurs on a variety of water sorted and
wind drifted glacial material, in some localities calcareous, in others
lacking in lime. These instances suggest that, relative to the pres-
ence or absence of lime in the rocks composing the drift and in the
chemical composition of the underlying mineral soils, differences of
that kind do not determine the extent, thickness, or parallelism in
the layering of the widely separated peatlands. The effects of differ-
ences in quantity and quality of salt content, especially in lime,
become observable as a rule after drainage, and more particularly
under cropping systems which favor evaporation of ground waters
at the surface. WHITSON (30) found that marshes which are acid
become well supplied with lime by the waters percolating through

the soils and rocks of the surrounding region. ALWAY (1) states that burning surface layers of peat in some cases may produce an alkali soil.

It would lie outside the limits of this paper to provide further data necessary to establish the view that no direct connection appears to exist between the development of peat deposits, both in quality and thickness of different layers, and a high or low lime content of the glacial lake beds and their drainage waters. The inauguration of peat formation, as well as the sequence of peat layers, obviously must be regarded as being largely independent of the chemical composition of the mineral soils. Distinctions on the basis of acidity or lime content appear to be inadequate for purposes of classifying peat deposits. The evidence at hand does not indicate that these are the controlling influences in the origin and stratigraphic similarity between the peatlands of the two glacial lake beds.

3. Climate, more particularly the climate of the past as a determining factor in the origin and structural features of peatlands, has only recently received the impartial consideration which it deserves. In Europe the development of this viewpoint by BLYTT (2), SERNANDER (23), and others has again received strong support by the more recent work of GAMS (13), RUDOLPH and FIABAS, and others.

The ice retreat in this country and in Europe does not appear to have been very different. The halt and readvance of the ice edge reflect climatic fluctuations, and when the morainal belts of the respective countries are properly matched, they show agreements in several respects (8).

The recession of the ice sheet marks the beginning of many peatlands in the glaciated regions of this country. In the western states the ice edge stood practically still for a considerable period of time, while it receded in the southeastern part of the country. The explanation is that probably in Minnesota and Wisconsin the summers were cold, the air was still very foggy, and the temperature low and constant in a belt off the ice front, while in the Ohio-Indiana region the sky was clear, and warm days were frequent. At that time, as today, the most important among the elements of climate in relation to types of vegetation, and to the quantity and character of peat

derived from them, must have been moisture from precipitation, and temperature. In the course of time the warmer zone shifted northward and the ice front retreated. As the surface waters of glacial lakes Agassiz and Wisconsin became reduced by run off and evaporation, the extent of peat accumulation over the uncovered land varied from time to time. The structural differences, as shown by the evidence presented in the profile sections, undoubtedly correspond in a general way with climatic changes. Precipitation may have played the leading part, since as a rule the amount of water shows greater effects upon peat formation in a topographically similar area, than does temperature. The conditions at the ice front had become milder and more stable when the basal conifer forest, essentially similar to the present vegetation, invaded the dry portion of the lake beds. After a time interval of considerable length, the physical conditions under which the forest trees grew ceased to exist. Changes in drainage, water supply, and atmospheric relations (again cooler and probably more humid) resulted in a new set of environmental surroundings, to which sedges and reed marshes, and in places *Hypnum* mosses, were better fitted to dominate. The presence of *Hypnum* peat seems to record a temporary readvance of the ice. The layer of buried forest is not always sharply differentiated from the fibrous peat material which formed above it. The vertical limits at times are difficult to distinguish; they evidently correspond to a gradual change in physical conditions.

Upon the surface of the sedge formed peat layer, a conifer forest once more made its appearance and began to spread over the open marshes. Essentially more favorable climatic conditions, warmer and drier, and related soil conditions prevailed, under which the forest vegetation of the conifer belt could grow and extend itself over peatlands. Reforestation and the accumulation of woody peat were again the characteristic features when the first settlers appeared. These conditions are in sharp contrast with the moisture relations which favored the long continued dominance of marsh vegetation. They must have been notably affected by lighter, more moderate rainfall; better aeration and decomposition must have taken place in the surface horizon of the peat soil.

## Summary

In conclusion, it should be stated that it is the climatic aspect of peat profiles which needs to be emphasized also in matters relating to the agricultural uses of these lake-bed peatlands. From the foregoing considerations it is evident that the present native surface vegetation is no indicator, either of the depth of peat accumulation, the sequence of layers, the character of the mineral subsoils, or the production possibilities for different systems of cropping. The profile sections indicate, furthermore, that production in these peat areas may depend chiefly on the quantity of water available. During the period before settlement, the arable land resources of the states had become filled with ground water from a long accumulation of rainfall and thaw waters. Since the settlement of the states, the ground water level has been steadily sinking. A serious situation may arise, therefore, in periods of drought, and settlers in that region may not be able to endure the years of adversity, if an extensive system of drainage is adopted without reference to the stratigraphic features of the peatlands. The provision of an adequate water supply and the control of water levels is essential if the agriculture of the region is to be permanent and satisfactory. The lack of a commensurate water supply may operate entirely against increasing production by intensive methods of farming. Such portions of the forested peatland area which contain deep, water logged depressions are important water storage basins. They would help to regulate and maintain a favorable ground water table. It seems far more profitable, therefore, to select such localities, and not to drain them, but to keep them as part of a state reserve of reforestation.

U.S. DEPARTMENT OF AGRICULTURE
     WASHINGTON, D.C.

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# NEW OR OTHERWISE NOTEWORTHY
## COMPOSITAE.  II

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 341

### EARL EDWARD SHERFF

### (WITH PLATES XIX–XXII)

In a former article[1] were discussed various specimens of *Bidens*, *Coreopsis*, *Cosmos*, *Isostigma*, etc., which had been found either to represent new species or to be otherwise important. Most of the specimens there cited were in the Herbarium of the Botanical Garden of Berlin. In the summer of 1924 I was privileged to visit Europe and examine many specimens of these genera. Some of the noteworthy specimens of *Isostigma* and many of those of *Bidens* will receive separate treatment in two forthcoming articles. Much of the other material, found chiefly in Berlin, Geneva, Paris, London, and Kew, will be considered here.

**Coreopsis senaria** Blake & Sherff, sp. nov.—Frutex; ramuli angulati lineatim hispiduli, internodiis brevibus; folia opposita brevia usque ad petiolos brevissimos connatos palmatim 3-partita, segmentis anguste lineari-oblanceolatis ca. 7 mm. longis 0.8 mm. latis; capitula solitaria in pedunculis brevissimis hirtellis insidentia; phyllaria interiora 8–9 exterioribus ca. 18 biseriatis lanceolatis ciliatis dimidio longiora; achaenia piloso-ciliata; aristae sursum ciliatae.

Shrub; branches 6-angled, rather slender, covered below with the persistent leaf bases, hispidulous in 2 lines; internodes 5–10 mm. long or less; leaves opposite, with axillary fascicles; petioles 1–1.5 mm. long, connate throughout their length, glabrous; blades palmately 3-parted to base, the lobes narrowly linear-oblanceolate, 6–8 mm. long, 0.6–0.9 mm. wide, with acute, callous tips,' entire, fleshy, glabrous, dark green, 1-nerved, veinless; peduncles solitary at tips of branches and the few branchlets, monocephalous, hirtellous, few-bracted, 1 cm. long or less; heads about 2 cm. wide; disk

[1] BOT. GAZ. 76:78. 1923.

about 7 mm. high, 9 mm. thick; involucre 7.5 mm. high, the outer phyllaries about 18, distinctly 2-seriate, subequal, herbaceous, lanceolate or linear-lanceolate, obtuse to acutish, often apiculate, 3-nerved, ciliate about to middle, glabrous dorsally, 4–5 mm. long, 1 mm. wide, the inner 8–9, membranous, oblong, obtuse, brown with narrow yellow margin, ciliolate, otherwise glabrous, 2.5 mm. wide; rays (probably 8) golden yellow, neutral, the tube glandular-puberulous above, 1.8 mm. long, the lamina oblong-oval, about 6 mm. long, 3 mm. wide, 11-nerved; disk corollas yellow throughout, sparsely puberulous toward apex of tube, 3.9 mm. long (tube 1.3 mm., throat slenderly campanulate-funnelform, 1.8 mm., teeth ovate, 0.8 mm.); pales lanceolate, 4 mm. long, acuminate, ciliate, pilose along midline, 5-nerved; disk achenes (very immature) obovate-oblong, pilose-ciliate on margin and at apex, glabrous on outer face, somewhat pilose on inner; awns 2, lanceolate, upwardly pilose-ciliate, 2.2 mm. long.

PERU: On punas, collected on an excursion from Pacasmayo to Moyobamba, April to June, 1875, *A. Stübel* 35 p.p. (type in Herb. Berl.; photog. and fragm. in U.S. Nat. Herb. no. 1,194,718).

Nearest *Coreopsis foliosa* Gray, but very distinct from that and from all other South American species in its leaves, which are palmately 3-parted down to the apex of the petiolar sheath. The species is unique also in its numerous and narrow outer phyllaries.

This and the next five species, all from South America, were submitted to SIDNEY F. BLAKE, Associate Botanist of the Bureau of Plant Industry, Washington, D.C., for examination. He had already made a critical study of the South American species of *Coreopsis* and was quick to recognize these as new species.

**Coreopsis parviceps** Blake & Sherff, sp. nov.—Frutex glaberrimus; rami angulati; folia opposita petiolata, inferiora 2–4-partita lobis linearibus vel lineari-ellipticis, superiora integerrima lineari-elliptica; capitula parva solitaria breviter pedunculata; involucri 6 mm. alti phyllaria interiora 8 oblonga glabra exterioribus 6 linearibus glabris duplo longiora; achaenia piloso-ciliata; corollae disci aristis ciliatis dimidio longiores.

Trichotomous shrub, essentially glabrous throughout; stem terete, striatulate, gray-barked; branches and branchlets slender, striate-angled, brownish; internodes 1.5–4 cm. long; petioles slender, 3–8 mm. long, connate into short cups, these slightly ciliolate in the

sinuses; lower blades deltoid in outline, about 2.2 cm. long, 2.8 cm. wide or less, palmately 2–3-parted or sometimes with 2-parted terminal lobe, thickish, glabrous, dark green on both sides, subpuncticulate, the lobes linear or linear-elliptic, 9–16 mm. long, 1.8–3 mm. wide, acute, translucently feather-veined; upper blades entire or sometimes 3-lobed, linear-elliptic, 8–19 mm. long, 1.5–3 mm. wide; peduncles solitary at tips of branches and branchlets, monocephalous, slender, glabrous, about 2.7 cm. long; heads 1.8 cm. wide; disk 6 mm. high and thick; involucre 6 mm. high, the outer phyllaries 6, thick-herbaceous, linear-oblong, 3 mm. long, 0.8 mm. wide, obtuse, glabrous, the inner 8, membranous, elliptic-oblong, 6 mm. long, 2.5 mm. wide, obtuse, glabrous, erose at apex, yellow with many brown nerves; rays 8, neutral, golden yellow, the tube pilosulous, 1.5 mm. long, the lamina oval, 7.5 mm. long, 4 mm. wide, 12-nerved, sub-entire; disk corollas rather few, golden yellow, sparsely puberulous at apex of tube, 3.5 mm. long (tube 1 mm., throat campanulate-funnelform, 1.6 mm., teeth ovate, 0.9 mm.); pales oblong-obovate, 3.5 mm. long, truncate or emarginate and somewhat spinulose-ciliate at apex, sparsely pubescent dorsally, about 5-nerved; ray achenes (inane) oblong, pilose-ciliate, sparsely pilose inside on midline; disk achenes (very immature) oblong, pilose-ciliate, ciliate at apex, pilose inside along midline; awns 2, lanceolate, upwardly pilose-ciliate, 2 mm. long; style tips deltoid-triangular, acuminate, hispidulous.

PERU: Tambillo, August 19, 1878, *de Jelski* 765 (types in Herb. Berl.; photog. and fragm. in U.S. Nat. Herb., no. 1,194,717).

Nearest *Coreopsis foliosa* Gray and *C. glaucodes* Blake & Sherff. The former has densely pilosulous and densely leafy branches, 3-parted leaves with lobes 1.2–2 mm. wide (the terminal one 3-partite), and oblong, rather densely pilosulous outer phyllaries. The latter is glaucous, with less deeply cut leaves and very narrow outer phyllaries.

**Coreopsis glaucodes** Blake & Sherff, sp. nov.—Frutex glaberrimus, partibus novellis glaucescentibus; folia opposita ambitu saepius cuneata vel rhombica ad medium vel ultra trilobata, lobis integris vel terminali tridentato oblanceolatis vel lanceolatis acutis; capitula parva 1–4 ad apices ramorum; involucri 4–6 mm. alti phyllaria interiora 8 exterioribus 8 anguste triangularibus duplo longiora; achaenia piloso-ciliata aristis sursum ciliatis duplo longiora.

Shrub 1 m. high, trichotomously branched, glabrous through-
out; stem terete, grayish-barked; branches and branchlets striate,
scarcely angled, brownish, glaucescent; internodes 1–6 cm. long;
leaves opposite; petioles slender, 4–10 mm. long, connate at base
into a cup about 1 mm. high; blades usually cuneate or rhombic in
outline, or lowest deltoid, 1.1–2.5 cm. long, 6–18 mm. wide, cuneate-
ly decurrent on petiole, usually 3- (rarely 4-) lobed about to middle or
rarely nearly to base, lobes cuneate or oblanceolate to linear-lanceo-
late or lanceolate, mostly 6–16 mm. long, 1.5–3.5 mm. wide, acute,
entire or terminal one (rarely also lateral) 2–3-dentate, thick, pale
green; glaucescent; peduncles 1–4 at tips of branches, monoce-
phalous, slender, glaucescent, usually naked, 1.5–5.5 cm. long; heads
1.2–2 cm. wide; disk 5–7 mm. high, about 7 mm. thick; involucre
4–6 mm. high, glabrous, glaucescent, outer phyllaries 8, herbaceous,
narrowly triangular, obtuse, 3-nerved, 2–2.5 mm. long, 0.2–0.5 mm.
wide, inner 8, submembranous, oblong, obtuse, obscurely ciliolate
at apex, many-nerved, yellow-margined, glaucescent, about 1.8 mm.
wide; rays 8, "orange yellow," neutral, tube sparsely pilosulous at
apex, 1 mm. long, lamina oblong, subentire, 7.5 mm. long, 3.2 mm.
wide, 8-nerved; disk corollas yellow, sparsely pilosulous at apex
of tube, 3.2 mm. long (tube 1 mm., throat campanulate-funnel-
form, 1.6 mm., teeth ovate, 0.6 mm.); pales oblong, acutish
or obtuse, about 5-nerved, pilose along midline, erose-spinulose
at apex, 4.5 mm. long; ray achenes (inane) linear, sparsely pilose-
ciliate; disk achenes obcompressed, obovate-oblong, 3.5–4 mm.
long, 1.8 mm. wide, pilose-ciliate, ciliate at apex, sparsely pilose
on midline inside; awns 2, lanceolate, upwardly ciliate, 1.5 mm.
long; style tips deltoid, hispidulous, apiculate.

PERU: In the open formation, below Hacienda La Tahona, near Hual-
gayoc, Dept. Cajamarca, altitude 2600 m., May 15, 1904, *Weberbauer* 4048
(types in Herb. Berl.; photog. and fragm. in U.S. Nat. Herb., no. 1,194,714).

Related to *Coreopsis parviceps* Blake & Sherff, but decidedly glaucescent on
the younger parts, and with less deeply divided leaves and much narrower
outer phyllaries.

**Coreopsis microlepis** Blake & Sherff, sp. nov.—Frutex subglaber,
caule tenui striato-angulato saepius simplice; folia opposita pe-
tiolata, lamina ambitu deltoidea paene ad costam trilobata, seg-
mentis lateralibus cuneatis vel elliptico-obovatis integris vel 2–4-

dentatis apiculatis, terminali saepius ad medium 3-lobato; capitula mediocria 3-15 cymam vel paniculam nudam efficientia, rarius solitaria; involucri 6-7 mm. alti phyllaria exteriora ca. 8 ovata obtusiuscula glabriuscula interioribus triplo breviora; achaenia pilosa et ciliata aristis lanceolatis ciliatis triplo longiora.

Shrub 40 cm. high and more; stem usually simple below the inflorescence, rarely with few simple erect branches, 1.8 mm. thick or less, 6-angled, glabrous or slightly hispidulous in lines below the nodes, inflorescence rather sparsely hispidulous; internodes 1.3-5 cm. long, usually longer than leaves; leaves opposite, often with fascicles in their axils; petioles hirsute-ciliate chiefly toward base, slightly margined above, 4-10 mm. long, connate at base into a cup; blades deltoid in outline, 8-21 mm. long, 7-22 mm. wide, coriaceous, glabrous, somewhat thickened on margin, 3-lobed nearly to midrib (rachis 1 mm. wide or less), lateral segments cuneate, elliptic-obovate, or spatulate, 5-11 mm. long, 2-6 mm. wide, terminal segment 3-4-lobed usually about to middle, lobes entire or 2-3-toothed; upper leaves smaller, those subtending the branches of the inflorescence usually very small, subulate, entire; heads rather small, usually 3-15 in terminal cyme or panicle, on pedicels 1.5-5 cm. long; disk 6-7 mm. high, about 9 mm. thick; involucre 6-7 mm. high, glabrous or somewhat hispidulous at extreme base, outer phyllaries 8-9, ovate or oblong-ovate, thick-herbaceous, obtusish, 1.5-2 mm. long, 0.6-0.8 mm. wide, the inner 8, submembranous, elliptic-oblong, obtuse, about 2.2 mm. wide, blackish with narrow yellow margins; rays probably 8, neutral, golden yellow, tube sparsely pilose, 1.2 mm. long, lamina oval, subentire, 7-nerved, sparsely hirsutulous dorsally on chief nerves, 5.5 mm. long, 3 mm. wide; disk corollas golden yellow, glabrous, 3.3 mm. long (tube 1.1 mm., throat funnelform, 1.6 mm., teeth ovate, papillose-margined, 0.6 mm. long); pales obovate, obtuse to acute, about 6-nerved, pilose along midline, 4.5 mm. long; ray achenes (inane) linear, epappose, pilose-ciliate, glabrous on faces; disk achenes obcompressed, obovate to oblong, 2.8-3.5 mm. long, about 1.2 mm. wide, long-pilose-ciliate, pilose on both faces; awns 2, lanceolate, subpaleaceous, pilose-ciliate, persistent, 0.8-1.2 mm. long; style tips broadened, hispidulous, abruptly apiculate.

PERU: Province of Chachapoyas, 1835-6, *Mathews* 1418 (types in Herb. Kew; photog. and fragm. in U.S. Nat. Herb., no. 1,198,100).

Based on two sheets in the Kew Herbarium bearing six specimens, accompanied by several different labels, but all collected by *Mathews* in Chachapoyas and all clearly conspecific. The nearest allies of this species are *Coreopsis foliosa* Gray, which is similar in cutting of leaves, but has densely spreading-pilosulous stems and oblong rather densely pilosulous outer phyllaries 3.5-5 mm. long (GRAY's types, in the Kew Herbarium, examined); *C. glaucodes*, which is glaucescent and has less deeply lobed leaves with fewer lobes, and very narrowly triangular outer phyllaries about half as long as the inner; and *C. parviceps*, which has fewer segments in the lower leaves, linear-elliptic and entire upper leaves, and linear-oblong outer phyllaries half as long as the inner. The specific name of the new species refers to the very small outer phyllaries.

**Coreopsis polyactis** Blake & Sherff, sp. nov.—Frutex densissime foliosus, ramis hirsutulis; folia opposita petiolata parva tripartita, segmentis saepius linearibus integris vel terminali tripartito; capitula brevissime pedunculata saepius solitaria majuscula; involucri ca. 1 cm. alti dense flavescenterque subtomentoso-pilosuli phyllaria interiora 13 exterioribus 11 ovatis distincte biseriatis subdimidio longiora; radii ca. 12; achaenia et aristae sursum ciliatae.

Shrub 1 m. high, trichotomous, densely leafy; branches subterete, sordid-hirsutulous chiefly in lines; internodes 4-10 mm. long; leaves opposite, usually with axillary fascicles; petioles 4-6 mm. long, somewhat ciliate, connate at base into hirsutulous cups 1 mm. high; blades deltoid in outline, 6-10 mm. long and about as wide, 3-parted, segments entire or terminal 3-parted, linear or narrowly linear-oblanceolate, 3-6 mm. long, 0.5-1 mm. wide, acute, thick, dark green, sparsely hirsutulous especially on margin; peduncles 1-3 at tips of branches, monocephalous, stout, densely and flavescently subtomentose-pilose, 1-1.8 cm. long, fistulose in age; heads 3-3.5 cm. wide; disk 1 cm. high, about 1.7 cm. thick; involucre 1-1.1 cm. high, densely and flavescently subtomentose-pilosulous, the outer phyllaries 11, distinctly biseriate, subequal, herbaceous, oval-ovate, obtuse, many-nerved, 6 mm. long, 3.3-4.8 mm. wide, the inner 13, thick-membranous, elliptic-oblong, narrowed to an obtuse or acutish apex, narrowly pale-margined, many-nerved; rays about 12, neutral, golden yellow, tube sparsely puberulous at apex, 2.5 mm. long, lamina oval, subentire, 10-nerved, 1.3 cm. long, 6.5 mm. wide; disk corollas yellow, apparently brownish-tipped, glabrous, ca. 4 mm.

long (tube 1.3 mm., throat funnelform, 2 mm., teeth ovate, 0.8 mm.);
pales linear-oblong, 6 mm. long, acute, about 7-nerved, sparsely
ciliolate above, pilose along midline; ray achenes (inane) glabrous,
epappose; disk achenes (immature) linear-oblong, obcompressed,
pilose-ciliate, pilose over whole inner face, glabrous on outer, not
ciliate at apex; awns 2, lanceolate, upwardly ciliate, 3–4 mm. long;
style tips deltoid, hispidulous, apiculate.

PERU: On grass steppes with scattered shrubs, between Hacienda Llaguén
and Succhabamba, Dept. Libertad, Prov. Otuzco, altitude 3500–3600 m., June
28, 1914, *Weberbauer 6995* (types in Herb. Berl. photog. and fragm. in U.S. Nat.
Herb., no. 1,194,716).

Distinct from all other South American species in its broadly oval-ovate,
biseriate outer phyllaries.

**Coreopsis notha** Blake & Sherff, sp. nov.—Frutex; rami striati
sparse hispiduli; folia opposita ternatisecta, segmentis lateralibus
3–5-sectis terminali longiusculo stipitato pinnatim 5-secto, seg-
mentis ultimis lanceolatis vel oblongis; capitula majuscula longe
pedunculata; phyllaria interiora 8 elliptico-oblonga glabra exteriori-
bus 6 oblongis basi ciliatis 4–5-plo longiora; achaenia piloso-ciliata
aristis ciliatis duplo longiora.

Shrub 1 m. high, with long branches; stem striatulate, subterete,
glabrescent or glabrate; branches striate-angulate, sparsely hispidu-
lous; internodes mostly 1–4.5 cm. long; petioles sparsely ciliate,
1–1.5 cm. long, connate at base into somewhat hispidulous cups 1.5
mm. high; blades deltoid in outline, 1.8–2.7 cm. long and wide, ter-
natisect, basal segments short-stipitate or subsessile, 3–5-sect, the
terminal long-stipitate, pinnately 5-sect with usually entire divisions,
blade thickish, dark green, obscurely pubescent and ciliolate, ulti-
mate segments lanceolate or oblong, acute, mostly 3–8 mm. long,
1–2.5 mm. wide; peduncles 1–3 at tips of stem and branches, 1
(rarely 2)-headed, sordidly crisped-pilosulous especially toward
apex, naked or with few small bracts, 7–21 cm. long; heads 3.4–
3.8 cm. wide; disk 7–8 mm. high, 8–11 mm. thick; involucre 9–12
mm. high, outer phyllaries constantly 6, herbaceous, oblong, 2–3
mm. long, 1–1.6 mm. wide, obtuse or rounded, somewhat ciliate at
base, otherwise glabrous outside, inside sordid-pilosulous except
toward apex, loose, inner phyllaries 8, membranous, oblong-elliptic,

9–12 mm. long, 3–4.6 mm. wide, obtuse, narrowly yellowish-margined, finely ciliolate at apex, otherwise glabrous; rays 8, neutral, "orange yellow," tube hispidulous, 2 mm. long, lamina oval, 1.5–2.1 cm. long, 9 mm. wide, subentire, 13–15-nerved; disk corollas yellow, hispidulous on tube and toward base of teeth, 5.8 mm. long (tube 1.8 mm., throat funnelform, 3 mm., teeth ovate, 1 mm.); pales oblong, 6–7 mm. long, hispidulous-ciliate at the truncate apex, pilose along midline of back, about 12-nerved; ray achenes (inane) linear, pilose-ciliate, sparsely pilose inside along midline; disk achenes linear-obovate, 6 mm. long, 1.7 mm. wide, obcompressed, pilose-ciliate, ciliolate at apex, pilose along midline inside; awns 2 (rarely 3), lance-linear, upwardly pilose-ciliate, 2.7–3.4 mm. long; style branches short-deltoid, hispidulous, apiculate.

PERU: Above San Pablo, Dept. Cajamarca, Prov. Cajamarca, altitude 2400–2700 m., April 26, 1904, *Weberbauer* 3812 (types in Herb. Berl.; photog. and fragm. in U.S. Nat. Herb., no. 1,194,719).

Nearest *C. spectabilis* Gray and *C. boliviana* Blake, but distinguished by its very short outer phyllaries and considerably broader ultimate lobes of leaves. Leaves similar to those of Mexican *C. rhyacophila* Greenm., but smaller and somewhat less dissected. Vernacular name is given as "pul," and the heads are said to be used for dyeing. As in all the other species here described (with the apparent exception of *C. glaucescens*, and perhaps also *C. microlepis*), the heads impart a rich orange color to the water in which they are boiled.

**Coreopsis elgonensis**, sp. nov.—Herba erecta, perennis, ramosa, 8–10 dm. alta; ramis angulatis, glabris, infra ligneis, internodis numerosis et saepe tantum 3–10 mm. longis. Folia opposita, numerosa, sessilia, tantum circ. 1 cm. longa, ternatim divisa, foliolis membranaceis, margine ciliatis, faciebus atro-punctatis et interdum sparsissime hispidis, cuneatis, ternatim lobatis vel integris, segmentis ultimis acerrime apiculatis, plerumque 2–3 mm. latis. Capitula pauca in corymbis disposita pedunculis 2–9 cm. longis, radiata, pansa ad anthesin 2.5–3.5 cm. lata et 8–11 mm. alta. Involucri bracteae exteriores 6–11, lineares, supra latiores, apice acutae, plerumque glabratae, 5–9 mm. longae; interiores lanceolatae, hispidae, paulo breviores. Flores ligulati 8–12, flavi, ligula anguste elliptico-oblanceolati, apice rotundati sed minute plus minusve denticulati, 8–12 mm. longi et 3–4.5 mm. lati. Achaenia late lineari-oblonga, plana, omnino atra, glabra, exaristata, 3.5–4.8 mm. longa et 1.1–1.3 mm. lata, marginibus non vere membranaceis.

R. A. *Dummer* 3304, locally frequent, in thicket, edge of cliff, west side of crater, alt. 13,000 feet, Mt. Elgon, Uganda, January, 1918 (type in Herb. Kew). A species strongly suggestive, in general habit, of certain South American species such as *Coreopsis polyactis* Blake and Sherff and *C. senaria* Blake and Sherff. The many small, sessile, cuneate, ternate or biternate leaves appear at first glance to be in whorls rather than in pairs. Correlated with the leaf abundance is the shortness of the internodes. Thus, for example, one branch of the type is seen to have 28 internodes in a length of 2.5 dm., giving an average length of only about 9 mm.

COREOPSIS BARTERI O. & H. Fl. Afr. Trop. **3**:390. 1877; *C. badia* Sherff, BOT. GAZ. **76**:90. 1923.

Recently I have had opportunity to compare the types of *Coreopsis Barteri* (Herb. Kew) and *C. badia* (Herb. Berl.) and found them to be identical. They are matched in turn by a third specimen, *W. B. Baikie*, banks of the Niger River, west tropical Africa (Herb. Kew). From the three specimens, the range is seen to extend from the Niger River southwest through Borgu (Bussango) into Togo.

COREOPSIS OCHRACEA O. Hoffm., Engler's Bot. Jahrb. **30**:431. 1901; *C. cosmophylla* Sherff, BOT. GAZ. **76**:90. *pl. 9. figs. h-n.* 1923; *Bidens ochracea* (O. Hoffm.) Sherff, *l.c.* 158.( pl. XIX).

HOFFMAN had not seen ripe fruits of this species.[2] In my own earlier studies it seemed that the specimens of this handsome species of the Nyassa region were of two kinds, differing generically on the basis of the winged character of the achenes. Further specimens have shown, however, that some material is merely more tardy than the rest in displaying achenial wings. As the specimens mature, their achenes become fairly well winged. Besides the type (*W. Goetze* 731, on red laterite, hilly plateau, in thin bush growth, at altitude of 1700 m., Bweni, Uhehe, March 11, 1899; Herb. Berl.), I have seen the following: *Ad. Stolz* 764, *pro parte*, growing 2 m. tall, at altitude of 600 m., Kyimbila, Langenburg, German East Africa, June 8, 1911 (Herb. Berl.); *idem* 764 *pro parte*, Kyimbila, at altitude of 600 m., February 27, 1912 (Herb. Deless.); *Muenzner* 159, Msamvia, Lake Tanganyika District, Germ. E. Afr., February 24, 1909 (Herb. Berl., type of *C. cosmophylla*); *A. Whyte*, northern Nyassaland (Herb. Kew); *E. Battiscombe* 83, at altitude of 3500–4000 feet, Muhoroni, Brit. E. Afr. (Herb. Kew; form with somewhat atypic foliage); *W. H. Nutt*, at about 6000 ft. altitude, between Lake Tanganyika and Lake Rukwa, in 1896 (Herb. Kew).

**Coreopsis feruloides**, sp. nov.—Herba erecta, ramosa, forsitan annua, circ. 7–10 dm. alta, glabra, viridis vel glaucescens, caule subtetragono. Folia sessilia vel subsessilia, 4–8 cm. longa, bi-

[2] "Reife Früchte fehlen; nach den Fruchtknoten zu erteilen könnten sie geflügelt sein"; Hoffm. *l.c.*

pinnata, costa mediana tenui, segmentis linearibus vel anguste lan-
ceolatis, crassiusculis, minute ciliatis, obtusis vel subacutis, ple-
rumque 2–4 mm. latis. Capitula subnumerosa, corymbosa, radiata,
pansa ad anthesin 2.5–3 cm. lata et 5–7 mm. alta. Involucri sub-
glabrati bracteae exteriores circ. 8, lineares, apice subobtusae in-
durataeque, 3–5 mm. longae, interiores lanceolatae circ. 6–7 mm.
longae. Achaenia matura minuta, non exserta, atra, valde obcom-
pressa, lineari-oblonga, leviter alata, faciebus subobscure striata,
faciebus marginibusque minute erecto-setosa, corpore tantum 3.5–
4 mm. longa et (alis inclusis) parce 1 mm. lata, apice biaristata;
aristis tenuissimis, stramineis, setulis minutis erectis numerosis
instructis, 1–1,6 mm. longis.

    *E. Battiscombe* 945, at altitude of 7500–8000 feet, Kinabop Plateau, Western
Aberdare Mountains, British East Africa (type in Herb. Kew).

    The general habit simulates very closely that of the Mexican *Bidens
grandiflora* Balb. and *B. ferulaefolia* (Jacq.) DC. The leaves suggest the pri-
mary lower lateral portion of the more decompound leaves of *Ferula glauca* L.
Aside from the habit, the tiny achenes are very distinctive, very few African
species of *Coreopsis* having such small ones. The achenial wings are of the same
color as the body proper, thus easily escaping notice. The species is somewhat
unwelcome, as it serves further to weaken the generic distinctions between
*Coreopsis* and *Bidens.*

    Cosmos sulphureus var. leiorhynchus Griseb., descript.
amplif.—Herba erecta, glabra, ± 6 dm. (verisimiliter usque ad 1 m.)
alta. Folia opposita, bipinnata vel tripinnata, principalia petiolo ad-
jecto 6–11 cm. longa, petiolis 1.5–3 cm. longis, foliolis linearibus, apice
acutis, margine integris sed minutissime spinuloso-ciliatis, non
crassis, 1–4 mm. latis. Capitula ramos terminantia, subtenuiter
pedunculata pedunculis 8–18 cm. longis, radiata, pansa ad anthesin
circ. 3–3.5 cm. lata et 8–12 mm. alta. Involucri bracteae glabrae,
exteriores circ. 8, lineari-subulatae, ad basim 1–1.5 mm. latae, e basi
usque ad apicem acutum induratumque sensim angustatae, 5–8 mm.
longae; interiores lanceolato-oblongae, paulo longiores. Flores ligu-
lati 6–8, flavidi, ligula ovato-oblanceolati, apice subdenticulati, 10–
14 mm. longi. Achaenia atra, lineari-fusiformia, obcompressa vel
tetragona, manifeste 4-sulcata, glabra, supra ad apicem truncatum
augustata, exaristata, 10–14 vel tantum 8 mm. longa et 1.5–2.2 mm.
lata, cervice non perspicua.

Liberty H. Bailey[3] and *Ethel Zoe Bailey* 509, Los Charros, Venezuela, alt. 3000–3500 feet, December 27, 1920 (Herb. Field Mus.); *Mary Strong Clemens* 801, Camp Keithley, Lake Lanao, Mindanao, Philippine Islands, November, 1906 (Herb. Field Mus.); *J. Linden* 1510, savannahs, alt. 3000 ft., Valencia, State of Carabobo, Venezuela, December, 1843 (Herb. Deless.)

This variety, the type of which was collected by Dr. CRUEGER on the Island of Trinidad (*fide* Grisebach Fl. Brit. W. Ind. Isls. 374. [1861 *pagina cit.*]), has various points of similarity to *Cosmos bipinnatus* Cav., *C. caudatus* H.B.K., *C. sulphureus* Cav., and *C. Landii* Sherff. It differs from the first and second in having, among other characters, yellow rays; from the third in having smooth, short-necked achenes and lemon yellow or golden yellow (not reddish yellow) ligules;[4] from the fourth in having leaves of membranaceous texture. The Philippine specimen, although coming from a place widely remote from the type locality, appears to differ very slightly from the Venezuela material. Its leaves are mainly tripinnate instead of mainly bipinnate, and its achenes are 8–10 mm. rather than 10–14 mm. long. I have not seen GRISEBACH'S type, but he cited also "Venezuela," and it may well be that he had seen the *Linden* plant collected there a few years before.

*Bidens calva* (Schz. Bip.) C. B. Clarke (founded upon *R. F. Hohenacker* 344, near city of Mangalor, East India, in 1847) is a form likewise with exaristate, but with longer and more rostrate achenes. It has been referred by J. D. HOOKER (Fl. Brit. Ind. 3:310. 1881) to *Cosmos sulphureus* Cav. The original specimens by HOHENACKER (Herb. Par., Herb. Deless., etc.), as well as several other sets of specimens examined by me, offer various intermediate aspects between typical *Cosmos sulphureus* Cav. and our plants, although the affinity with the latter is greater. Hence one cannot well assign to our plants, with their light yellow rays and exaristate, shorter, and less rostrate achenes, higher than varietal status.[5]

BIDENS PILOSA var. **calcicola** (Greenm.), comb. nov.; *Cosmos pilosus* H. B. K., Nov. Gen. et. Sp. 4:241 (189). 1820; *Bidens rosea* Schz. Bip. *in* Seem. Bot. Voy. Herald 308. 1852–1857; *B. exaristata* DC. Prodr. 5:600. 1836; *B. brachycarpa* DC. *l.c.; B. rosea* var. *calcicola* Greenm., Proc. Amer. Acad. 41:264. 1905; *B. pilosa* var.

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[3] I am indebted to Dr. BAILEY for having sent me this material.

[4] CAVANILLE'S employment of the name *sulphureus* was unfortunate. In the species *C. sulphureus* Cav. proper the rays are distinctly orange in color (*cf. C. aurantiacus* Klatt [Leopoldina 25:105 1889], founded upon ordinary specimens of *Cosmos sulphureus* Cav., *Gust. Bernoulli*, in campis ad Tacotenango prope urbem Guatemala, Guat., December) and not yellow, as properly stated by GRISEBACH for the var. *leiorhynchus.*

[5] For the synonym *Bidens artemisiaefolius* var. *calvus* (Schz. Bip.) O. Ktze., which is invalid according to the Vienna Code, see O. KUNTZE, Rev. Gen. Pl. 1:321. 1891.

*brachycarpa* (DC.) O. E. Schulz, Urban Symb. Antill. 7:138.
1911.—Pl. XX.

GREENMAN (*l.c.*) lists *Palmer* 192, *E. W. Nelson* 6868, and *Heyde* and *Lux*
6172 (all in Herb. Gray) as representing *Bidens rosea* Schz. Bip. (*Cosmos pilosus*
H.B.K.). Without discussing *B. rosea* Schz. Bip., the immature type of which
(in Herb. Par.) is especially well matched by the *Palmer* plant, it is sufficient
here to state that these specimens are referable to *Bidens brachycarpa* DC. The
type and cotype specimens of *B. brachycarpa* DC. (Herb. Prodr. in Herb.
Deless., Herb. Deless., Herb. Par., etc.), with their small leaves, small heads,
more or less rosaceous ligules, and small, exaristate, upwardly attenuate ach-
enes, appear widely different from typical *B. pilosa* L. A study of the various
other herbarium specimens cited later, however, shows it to be utterly impossible
to maintain separate specific rank for them; rather must they be given varietal
rank under *B. pilosa* as was done by O. E. SCHULZ (*l.c.*). According to the
Vienna Code, however, the earlier varietal name *calcicola* must be applied here.
The type of the var. *calcicola*, *Pringle* 11340 (Herb. Gray) has a single pair of
stem leaves present, these pinnately 5-parted, with the terminal and basal
leaflets being more or less definitely 3-parted; the divisions are lanceolate; the
involucres are rather densely canous-pubescent. These characters are found to
be duplicated in various of the specimens cited below.

DeCandolle appears to have attached undue importance to the amount of
scabridity on the achenial surfaces. Thus he placed *Berlandier* 5 and 113, with
achenes scabrous, in his new species *B. brachycarpa*, but *Berlandier* 2220, with
achenes less glabrous[6] and happening to have a taller, more corymbose habit,
with the terminal leaflets more elongate, he described separately under the new
name *B. exaristata*. The technical characters of flower and fruit offer no warrant
for maintaining *B. exaristata* as a separate species or variety apart from the var.
*calcicola*. As to the somewhat taller habit and more corymbosely or even
fastigiately branched inflorescence observed in certain specimens (*Berlandier*
39, Herb. Deless., Herb. Drake *in* Herb. Par.; *idem* 2220, Herb. Deless., Herb.
Drake *in* Herb. Par.; *Nelson* 2111, Herb. U.S. Nat.), these seem to be merely
the result of a capricious growth. Indeed, *Berlandier* 800 and 2220 in the Gray
Herbarium have a much more loosely corymbose inflorescence as, in fact,
DECANDOLLE's description states ("laxe corymbosa"), and thus at once remove
from consideration the only character that by some botanists might be thought
important. Through the first BERLANDIER specimen just cited (no. 800) an ap-
proach is made toward *B. pilosa* var. *bimucronata* (Turcz.) O. E. Schulz, a
variety which has the heads usually larger, the leaves more often glabrous or
subglabrous, and the lateral leaflets (in the variety proper) typically undivided.

Specimens examined: *Fr. Gerfroy Arsène*, alt. 2100 m., Punguábo, State of

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[6] DECANDOLLE (*l.c.*) actually described the achenes as glabrous ("achaeniis
glabris"), but in two sheets of *Berlandier* 2220 (Herb. Deless.) now before me, many
of the achenes are distinctly scabrous near the apex.

Morelia, Mexico, October 17, 1909 (Herb. Deless.); *Berlandier* 5 and 113, Tampico, State of Tamaulipas, Mexico, in 1827 (Herb. DC. Prodr.; Herb. Deless.; Herb. Par.; Herb. Webb); *idem* 39, Mexico (Herb. Par.); *idem* 800, Mexico (Herb. Gray); *idem* 2220, between Victoria and Tula, Mexico, November 1830 (Herb. DC. Prodr.; Herb. Deless.; Herb. Gray; Herb. Par.); *Bourgeau* 2253, Valley of Cordova, Mexico, April 16, 1866 (Herb. Par.); *Prof. A. Dugès*, in garden near Guanajuato, State of Guanajuato, Mexico, in 1891 (Herb. Gray); *Carl Heller* 37, alt. 3000 ft., in meadows, Mirador, State of Vera Cruz, Mexico (Herb. Par.; Herb. Mus. Vienna); *Heyde and Lux* 3788, alt. 3500 ft., Cerro Gordo, Dept. Santa Rosa, Guatemala, September 1892 (Herb. Mun.); *idem* 6172, alt. 800 m., Cuijiniquilapa, Dept. Santa Rosa, Guatemala, November 1893 (Herb. Boiss.; Herb. Brit. Mus.; Herb. Field Mus.; Herb. Gray); *Dr. H. Karsten*, Quindio, Colombia (Herb. Petrop.); *E. Kerber* 9, Cordoba, State of Vera Cruz, Mexico, July 22, 1882 (Herb. Boiss.; Herb. Brit. Mus.; Herb. Copenh.; Herb. Deless.; Herb. Mun.; Herb. Par., 3 sheets; Herb. Mus. Vienna, etc.); *Liebmann* 640, Colipa, State of Vera Cruz, Mexico, March 1841 (Herb. Copenh., 2 sheets); *idem* 641, Papantla, State of Vera Cruz, Mexico, June 1841 (Herb. Copenh.); *idem* 643, Mirador, State of Vera Cruz, January 1843 (Herb. Copenh.); *idem* 651, Tehuacan, State of Puebla, Mexico, December 1841 (Herb. Copenh.); *Fred Müller* 238, Orizaba, State of Vera Cruz, Mexico, in 1853 (Herb. N.Y. Bot. Gard.); *E. W. Nelson* 2111, alt. 4500–5700 ft., between Tlapa and Ayusinapa, State of Guerrero, Mexico, December 13, 1894 (Herb. Gray); *idem* 6868, Los Reyes, State of Michoacan, Mexico, February 8–12, 1903 (Herb. Gray, foliis glabris et valde membranaceis); *Fr. Nicholas*, bank of the Atoyac River near Puebla, State of Puebla, Mexico, July 15, 1909 (Herb. Mun.); *idem* 5, Teocalli de Choluca, State of Puebla, Mexico, December 1911 (Herb. Deless.); *Dr. Edward Palmer* 68, alt. circ. 15 m., vicinity of Tampico, State of Tamaulipas, Mexico, January 1910 (Herb. Mo. Bot. Gard.).

BIDENS PILOSA CALCICOLA **dissecta**, f. nov.—A varietate differt: foliis bipinnatis vel etiam tripinnatisectis, segmentis linearibus vel anguste lanceolatis.

Specimens examined: *Fr. Gerfroy Arsène* 11, alt. 2000 m., Loma S. Maria, State of Morelia, Mexico, September 4, 1910 (Herb. Deless., foliis glabratis, valde membranaceis); *Heyde and Lux* 6164, alt. 1300 m., Malpais, Dept. Santa Rosa, Guatemala, November 1893 (Herb. Berl., 2 sheets; Herb. Boiss., 4 sheets; Herb. Brit. Mus.; Herb. Copenh.; Herb. Field Mus.; Herb. Gray, type; Herb. Kew); *iidem* 6170, alt. 1600 m., La Vega, Dept. Santa Rosa, Guatemala, September 1893 (Herb. Berl.; Herb. Boiss.; Herb. Brit. Mus.; Herb. Gray; Herb. Kew); *Schaffner*, Mexico (Herb. Berl.); *Caec. and Ed. Seler* 1184, Patzcuaro, State of Michoacan, Mexico, November 2, 1895 (Herb. N.Y. Bot. Gard.).

A form comparable with the variety *calcicola* in much the same way that the bipinnately leaved form (to be discussed later) of *B. pilosa* var. *bimucronata* (Turcz.) O. E. Schulz is comparable with that variety. Indeed, of the var.

*bimucronata* and its form with more compound foliage, there are found at times stunted or dwarfed specimens which have flowering and fruiting heads diminutive enough to be taken for var. *calcicola* and f. *dissecta*. It is therefore possible that in some of my past herbarium determinations I may have confused the two varieties and their respective forms to a slight extent.[7]

BIDENS ANDICOLA **Mandonii**, var. nov.—A specie differt: saepius annua, altior (usque ad 8 vel 10 dm. alta), minus ramosa et magis attenuata, capitulis ad anthesin subradiatis, minoribus, tantum circ. 5 mm. latis et 5–7 mm. altis, achaeniis gracilioribus.

Specimens examined: *G. Mandon* 48 (type) and 44 *pro parte*, in uncultivated places, alt. 2650 m., vicinity of Sorata (San Pedro), Bolivia, March 1859 (Herb. Boiss.; Herb. Brit. Mus.; Herb. Deless.; Herb. Kew; Herb. Par.; Herb. Mus. Vienna).

M. GUSTAVE BEAUVERD, in 1919, had referred my type (Herb. Boiss.) doubtfully to a variety of *B. pilosa* L. The fruiting heads, however, are more those of *B. andicola* H.B.K. *Mandon* 44, collected at the same time and place (Herb. Brit. Mus., Herb. Kew, Herb. Mus. Vienna), offers several transitional forms that connect this variety satisfactorily with typical *B. andicola*. In fact the Boissier Herbarium specimen of *Mandon* 44 and one in the Museum at Vienna are closer to the variety than to the species proper.

BIDENS VULGATA **dissectior**, var. nov.—A specie differt: foliis principalibus bipinnatis vel tripinnatisectis.

*E. Bourgeau* (Palliser's Brit. N. Amer. Expl. Exped.), willow marsh at edge of Saskatchewan River, Saskatchewan, September 18, 1857 (Herb. Par., type; Herb. Kew).

BIDENS ANTHRISCOIDES **decomposita**, var. nov.—A specie differt: foliis bipinnatis.

*C. G. Pringle* 11822 *pro parte*, Barranca near Guadalajara, Jalisco, Mexico, October 17, 1903 (Herb. Kew, type; Herb. Berl.; Herb. Gray; Herb. U.S. Nat.).

*Pringle* 11822 in the Herbarium of Field Museum has the leaves tripartite and matches the type material of *Bidens anthriscoides* DC. (*Berlandier* 1010, Herb. Deless.; Herb. Brit. Mus.; Herb. Par.) fairly well except in being glabrate. Elsewhere, the specimens of *Pringle* 11822 are seen to have delicately bipinnate leaves, similar to those of *Bidens odorata* Cav. Through this form, apparently best regarded as a variety, *B. anthriscoides* DC. is found to be rather closely related to *B. inermis* Wats.

BIDENS REMYI (Hillebr.) Sherff, BOT. GAZ. **70**:97. 1920; *Campylotheca Remyi* Hillebr. Fl. Hawaiian Isls. 212. 1888; *Coreopsis*

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[7] For var. *calcicola* and f. *dissecta* I have, in the various herbaria, used previously the name *B. pilosa* var. *brachycarpa* (D.C) O. E. Schz., etc.

*Hillebrandiana* Drake del Cast. Illustr. Fl. Ins. Mar. Pacif. 209. 1890; *Campylotheca rutifolia* Lévl., Fedde Repert. Spec. Nov. **10**: 123. 1911.

LÉVEILLÉ's *Campylotheca rutifolia* was based upon *Abbé Urbain Faurie* 931, Wailau, Molokai, Hawaiian Islands, June 1910 and *idem* 965, Hawaiian Islands, in 1909. Both of FAURIE's numbers have been examined by me (no. 931, Herb. Brit. Mus. and Herb. Par.; no. 965, Herb. Brit. Mus.). They match perfectly the type material (Herb. Gray) of *Bidens Remyi* (Hillebr.) Sherff, to which they must be referred.

BIDENS MAUIENSIS **lanaiensis** var. primum nominat.; *Campylotheca mauiensis* var. β (*sine nom.*) Hillebrand Fl. Hawaiian Isls. 213. 1888.—Var. foliis validius membranaceis, saepe validius divisa, petiolis tenuibus usque ad 5 cm. longis; capitulis minoribus, pansis ad anthesin circ. 1.2–1.5 cm. latis; bracteis exterioribus minoribus; floribus ligulatis plerumque tantum 5 vel 6; achaeniis brevioribus 5–7 mm. longis, atris, plano-convexis vel etiam subtetragonis, nunc anguste alatis nunc exalatis, apice exaristatis sed minute coronulatis.

*Dr. William Hillebrand*, Island of Lanai, Hawaiian Islands, in 1879 (Herb. Gray, type); *idem*, Lanai, July 1870 (Herb. Brit. Mus.; Herb. Kew); *idem*, northern Maui, Hawaiian Islands (Herb. Brit. Mus.); *G. C. Munro* 450 and 451, Maunalei, Lanai, April 19, 1915 (Herb. Brit. Mus.).

The specimens from Lanai, as also the *Hillebrand* specimen from "northern Maui," are of the same general habit as the typical *B. mauiensis* (Gray) Sherff from Maui, but in respect to the characters noted are definitely marked. It will be observed that there are more pronounced differences than are found to occur in certain other cases between two accepted species (for example, *Bidens connata* Muhl. and *B. comosa* [Gray] Wieg.; *B. pilosa* L. and *B. chinensis* [L.] Willd.). In the case at hand, however, the remarkable degree of endemism displayed by Hawaiian plants renders the value of these distinguishing characters somewhat uncertain. For the present, I have thought it wisest to follow the treatment of HILLEBRAND and also of the late C. N. FORBES (*cf.* SHERFF, BOT. GAZ. **70**:98. 1920), both of whom regarded the Lanai material as representing a variety or varieties of *B. mauiensis*.

COSMOS PURPUREUS (DC.) Benth. and Hook. Gen. Pl. **2**:387. 1876; Hemsl. Biol. Centr. Amer. Bot. **2**:200. 1881; *Bidens purpurea* DC., *cum* var. *glabriuscula* DC. Prodr. **5**:604. 1836; *Coreopsis purpurea* Moc. Sess. ex DC. *l.c.*; *Cosmos Uhdeanus* Kunth, Ind. Sem. Hort. Berol. 1846 Coll. 12; Walp. Repert. **6**:721. 1847.

The original material of *Cosmos Uhdeanus* Kth. has not been seen by me, but the description is fairly satisfactory and leaves no doubt as to which of the species of *Cosmos* was intended. Robinson (Proc. Amer. Acad. 44:623. 1909) has properly associated with this name *Pringle* 8238, a handsome plant liberally represented in the larger herbaria, and matched, further, by a beautiful specimen collected more recently by Adole at Vera Cruz (Herb. Field Mus.).

*Bidens purpurea* DC. and its var. *glabriuscula* DC.[8] were founded respectively upon *Berlandier* 1007 and *Berlandier* 1021 [*pro parte*]. These numbers, also *Berlandier* 985 and 1163, are especially well represented in the valuable herbarium left by Moïse Etienne Moricand (and now in Herb. Deless.). Excluding one sheet of no. 1021, which is typical *Cosmos scabiosoides* H.B.K., the rest show various intergradations from small leaves 2–3 cm. long, pinnatisect as described by DeCandolle, to larger leaves 6–8.5 cm. long, definitely bipinnate and matching those of the *Pringle* and the *Adole* plants. Thus *Cosmos Uhdeanus* Kunth, typified as it appears to be by *Pringle* 8238 and by the *Adole* plant, is found synonymous with and referable to *Cosmos purpureus* (Kth.) B. & H.

From *Cosmos purpureus*, *C. scabiosoides* is seen to differ in having the rays usually (though by no means always) a darker purple, often almost black; the disk florets bright yellow except for the purple teeth (in *C. purpureus* the disc florets are entirely dark purple or sometimes yellowish merely at the base); the mature achenes long-attenuate above, not obscurely cervicate or even of full thickness; the less compound leaves dull-colored, apparently thickish, not thinnish, not light bright green above nor tending to a reddish brown below (against which, in *C. purpureus*, the white, stiff hairs appear, under a lens, in sharp contrast).[9]

Specimens examined: *Brother Adole*, Vera Cruz, Mexico, October 1910 (Herb. Field Mus.); *Berlandier* 985, Cordillera de Guchilaque, Mexico (Herb. Deless.); *idem* 1007, west of the Cordillera de Guchilaque, Mexico, October 1827 (Herb. Brit. Mus.; Herb. Deless., 3 sheets; type and cotypes); *idem* 1021 *pro parte*, Cordillera de Guchilaque, Mexico, October 1827 (Herb. Deless.); *idem* 1163, Toluca, State of Mexico, Mexico (Herb. Deless.); *C. G. Pringle* 8238, alt. 6500 ft., mountains above Cuernavaca, Morelos, Mexico, September 30, 1899 (Herb. Field Mus.; Herb. Univ. Vienna).

[8] The var. *glabriuscula* was merely a subglabrous variant and does not appear to merit segregation, even as a *forma*.

[9] Specimens of *Cosmos scabiosoides* H.B.K. recently examined: *Berlandier* 1021 *pro parte*, Cordillera de Guchilaque, Mexico (Herb. Deless.); *E. Bourgeau* 2930, Escamella, Vera Cruz, Mexico, September 1–2, 1866 (Herb. Deless., 2 sheets; Herb. Univ. Vienna); *C. A. Purpus* 1551, hillsides, open woods, alt. 7000–8000 ft., Salto de Agua, Mexico, November 1905 (Herb. Field. Mus; Herb. Univ. Vienna, 2 sheets); *C. G. Pringle* 4263, hills of Patzcuaro (type locality), Michoacan, Mexico, October 11, 1892 (Herb. Field Mus., 3 sheets; Herb. Univ. Vienna, simple-leaved state, f. *indivisus* Robinson); *idem* 9888, alt. 8500 ft., hills near Ozumba, State of Mexico, Mexico, November 4, 1902 (Herb. Field Mus.).

BIDENS TRIPLINERVIA H.B.K. Nov. Gen. et Sp. 4:231 (182).
1820; *B. serrata* Pav. *ex* DC. Prodr. 5:597. 1836.

To *Bidens triplinervia* H.B.K., which I have already discussed rather fully
(BOT. GAZ. **76**:155. 1923), must be referred *B. serrata* Pav. *ex* DC. The type
(no. 977 of the Ruiz, Pavon, and Dombey expedition, Peru, in 1788; *cf.*
LASÈGUE, Mus. Bot. Deless. 244–247. 1845) is extant in the DeCandolle
Prodromus Herbarium (Herb. Deless.). It lacks flowers, as stated by DE
CANDOLLE, but is seen to be merely a form of *B. triplinervia* with some leaves
undivided and lanceolate or narrowly oblong; others somewhat incised or lobed
at the base.

BIDENS TRIPLINERVIA var. **macrantha** (Wedd.), comb. nov.; *B.
glaberrima* DC. Prodr. **5**:601. 1836; *B. humilis* var. *macrantha* Wed-
del Chlor. And. 1:69. 1856.

In the long list of additional names already given by me (BOT. GAZ. **76**:155.
1923) as referable to *Bidens triplinervia* H.B.K., it is seen that the majority
refer to forms with leaves at least bipinnately compound. The simple-leaved
form, described under the names *B. triplinervia* H.B.K., *B. hirtella* H.B.K., *B.
procumbens* H.B.K., *B. serrata* Pav. *ex* DC., and *B. affinis* Kl. and O., is rather
rare.[10] The form with mostly bipinnate or tripinnate leaves is the one indicated
under *B. crithmifolia* H.B.K., *B. delphinifolia* H.B.K., *B. humilis* H.B.K., *B.
artemisiaefolia* Poepp. and Endl., *B. consolidaefolia* Turcz., *B. humilis* vars.
*macrantha* Wedd. and *major* Schz. Bip., *B. decomposita* var. *hirsutior* C. B.
Clarke, *B. humilis* var. *tenuifolia* Schz. Bip. *ex* Griseb., *B. pilosa* var. *discoidea*
subvar. *decomposita* f. *hirsutior* O. Ktze. (Rev. Gen. **1**:322. 1891), *B. grandiflora*

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[10] *B. hirtella* H.B.K. (Pl. XXI) was based upon a specimen from an unknown local-
ity, but with undivided though narrower leaves: "Folia opposita, petiolata, lanceolata,
acuta, serrata, basim versus subincisa, in petiolum angustata et integerrima, reticulato-
venosa, membranacea, utrinque hirta, subtus pallidiora, subpollicaria. Petioli duas
lineas longi, pubescenti-hirti, basi dilatati et connati . . . . folia nec triplinervia sunt."
KUNTH's type (Herb. Par.) does have the leaves with a distinct suggestion of two lateral
nerves running nearly half-way up the blade, giving a somewhat triplinervate appear-
ance. BONPLAND gave an extra specimen to the Paris Herbarium under the name *B.
hirtella* H.B.K. This was from Chillo, Ecuador ("Chillo, villa generosissimi Mar-
chionis de Selvalegre" H.B.K. *l.c.* 7:380. 1825) and the leaves exactly match those of
the type material of *B. triplinervia* H.B.K., not of *B. hirtella* H.B.K.

*B. procumbens* H.B.K. was described likewise from a narrower-leaved form:
"Folia opposita, petiolata, lanceolata, acuta, serrata, basi angustata et integerrima,
reticulato-venosa, subtriplinervia, membranacea, utrinque hispidula, subtus pallidiora,
adjecto petiolo subsesquipollicaria; inferiora multo minora, magis approximata. Petioli
subciliati." The type sheet (Herb. Par.) is accompanied by an extra sheet with one
small plant. This has leaves more ovate, approaching those typical for *B. triplinervia*,
the rays are about five as in the type, and the label says, "Bidens procumbens
varietas?"

var. *humilis* (H.B.K.) O. Ktze. (*l.c.* 3¹¹:136. 1898) and *B. attenuata* Sherff.¹¹ My recent examination of the type of *Bidens glaberrima* DC. (Herb. Prodr. in Herb. Deless.) revealed once again a mere compound-leaved form ("foliis oppositis petiolatis pinnato-multifidis intermedio multo longioribus, lobis oblongo-lineari-bus crassiusculis," DC. *l.c.*) of *B. triplinervia* H.B.K.¹²

A study of several hundred specimens of this species indicates such a wide range of variation in height of plants, amount of pubescence, form and size of leaves and size of flowering heads, that attempts at fine distinctions prove fruit-less. In a broad way, however, the species may be divided into three major divisions upon the basis of the simplicity or the compoundness of the leaves. The first, already mentioned for typical *B. triplinervia*, is characterized by hav-ing the leaves all or mainly simple, usually ovate or ovate-lanceolate, rarely linear-lanceolate. The second, apparently first described with varietal rank by WEDDELL (*l.c.*),¹³ is the second form just given, represented by the synonyms *B. crithmifolia*, *B. delphinifolia*, etc., and including *B. glaberrima* DC. It may be known as *B. triplinervia* var. *macrantha* (Wedd.). The third, like the first rather rare comparatively, has the leaves tripartite, with usually ovate and nearly always very pubescent leaflets. It thus stands, in leaf form, inter-mediately between the first two kinds. It is the *Bidens mollis* of POEPPIG and ENDLICHER (Nov. Gen. et Sp. 3:49. 1845), and may more properly be taken as:

BIDENS TRIPLINERVIA var. **mollis** (Poepp. and Endl.), comb. nov. (Pl. XXII figs. *a–i*)—Folia tripartita, saepius molliter pubescenti-villosa et subcanescentia, foliolis serratis, lateralibus plerumque ovatis, abrupte in basim sessilem contractis, terminali majore, ob-longo-ovato vel rhomboideo-ovato.

¹¹ Pl. XXII figs, *j–p*. At the time that *B, attenuata* originally was described (from Ghiesbreght 85, *pro parte*), *B. triplinervia* var. *macrantha* or its synonyms were com-monly restricted to South American specimens. I have since found abun-dant material from Guatemala (*Salvin*, Volcan de Fuego) and Mexico (*Ghiesbreght* 85, 533, etc., Chiapas; *Cuming* 53, Oaxaca; *Galeotti* 2021 and 2067, Oaxaca; *Pringle* 4915, Oaxaca; *Sallè* 91, Oaxaca; *Galeotti* 2169, Vera Cruz; *Linden* 491, Vera Cruz; *Palmer* 2062, Coahuila) which is inseparable from the South American material and shows a more northern range than formerly supposed. Among the Mexican specimens studied are several which display more or less pronounced transitions from the typical form to *B. attenuata* and reveal the latter to be merely an attenuated form with the five rays rather large and the almost flagelliform leaflets more or less convolute.

¹² It will be observed that *B. glaberrima* DC. is widely different from *Cosmos Landii* Sherff (*Bidens Palmeri* Gray) to which A. GRAY (Proc. Amer. Acad. 22:429. 1887) ap-parently had suspected it of belonging.

¹³ WEDDELL's type was *William Jameson* 55, alt. 13,000 ft., on rocks, Pichincha Mts., Ecuador, January 21, 1856. Certain other specimens by Jameson were 8-rayed, and thus approached somewhat *B. andicola* H.B.K. *Jameson* 55, however, is very definitely 5-rayed at Kew and at Geneva. In London (Herb. Brit. Mus.) one of the heads has 7 rays.

Specimens of var. *mollis* examined (partial list): *Gust. Bernoulli* 155, dry place, Calvario, Guatemala, November 1865 (Herb. Deless.); *F. C. Lehmann* 357, at edges of woods and open forest places, alt. 2000 m., Tunguragua River, Peru, October 31, 1879 (Herb. Boiss.; Herb. Kew); *idem* 433, alt. 2000 m., arid valley of Baños, Tunguragua River, Peru, December 13, 1880 (Herb. Boiss.); *idem* 1608, damp places, alt. 2000 m., San Marcos, Guatemala, June 17, 1884 (Herb. Boiss., more robust form 6 dm. high, the leaflets 3–5 cm. long); *idem* 2835, above Pais Camba on the Sotorá, alt. 2600–3000 m., Cauca, Colombia, May 6, 1883 (Herb. Berl.; Herb. Boiss.); *idem* 3511, alt. 2650 m., Cauca, Colombia, February 1, 1884 (Herb. Boiss.; Herb. Brit. Mus.); *G. Mandon* 43 *pro parte*, in uncultivated places, on hills etc., alt. 2600–2700 m., Espada Munaypata, San Pedro, vicinity of Sorata, Bolivia, March, 1859 (Herb. Deless., 2 sheets); *idem* 43 *pro parte*, everywhere in dry, rocky, uncultivated places etc., alt. 2650–3200 m., vicinity of Sorata, Bolivia, February–May 1859 (Herb. Boiss.; Herb. Brit. Mus.; Herb. Deless.; Herb. Par.; Herb. Mus. Vienna); *idem* 46 *pro parte*, slopes of hills, uncultivated places etc., alt. 2800 m., vicinity of Sorata, Bolivia, March 1859 (Herb. Boiss.; *alibi est* var. *macrantha*); Mathews, vicinity of Chachapoyas, Peru (Herb. Kew, form with achenes all exaristate; Herb. Deless., 2 sheets); *Eduard Poeppig* 1377, open, warm places, calcareous mountains at Cassapi, Peru, September 1829 (Herb. Mus. Vienna, type); *R. Spruce* 5047, at foot of Mt. Tunguragua, Ecuador, 1857–1859 (Herb. Boiss.; Herb. Brit. Mus.; Herb. Copenh.; Herb. Deless., 2 sheets; Herb. Kew, 2 sheets; Herb. Par.; Herb. Petrop.; Herb. Mus. Vienna).

**BIDENS SPECIOSA** var. **PATULA** (Gardn.) O. E. Schulz, Urban Symb. Antill. **7**:142. 1911; *B. patula* Gardn., Hook. Lond. Jour. Bot. **7**:405. 1848; *B. longipetiolata* Rusby, Bull. N. Y. Bot. Gard. **8**:131. 1912.

*B. patula* Gardn., correctly referred by O. E. SCHULZ to a variety of *B. speciosa*, differs from *B. speciosa* proper in having the leaves all simple, ovate-lanceolate, up to 5.5 cm. wide. *B. longipetiolata* Rusby (*R. S. Williams* 194, alt. 3800 ft., Mychariapo, Bolivia, April 9, 1902; Herb. Brit. Mus.; Herb. N.Y. Bot. Gard., type) is seen to be a form of the var. *patula*, differing in having the somewhat longer petioles measuring up to 4.5 cm. in length.

**Bidens Oerstediana** (Benth. *ex* Oerst.), comb. nov.; *Coreopsis Oerstediana* Benth. *ex* Oerst., Vidensk. Meddel. Kjöbenh. 1852:93. 1852.

This species from Nicaragua[14] was collected by OERSTED "in monte Masaÿa . . . . 1851." It does not appear to have been collected since then. In fact, the region around Masaÿa, in western Nicaragua, has been explored botanically very little. The type specimen at Kew[15] is a slender, herbaceous plant, quite un-

[14] Not Brazil, as stated in the Index Kewensis.

[15] The OERSTED plants, generously loaned to me from the Herbarium of the University of Copenhagen by Dr. CARL CHRISTENSEN, do not appear to have among them a duplicate of this species.

like the fruticose species so commonly found in Mexico and Central America for true *Coreopsis*. BENTHAM's original description likened it to some species of *Bidens*, but withheld it from *Bidens* because of the upwardly barbed achenial aristae. The young achenes were described as *subalata*. The type specimen, though slightly immature in its achenes, is of a plant related to *Bidens coronata* (L.) Britton (*B. trichosperma* Michx.) of the northeastern United States. Its achenes are somewhat immature, but are not more plainly margined or sub-winged than the average *Bidens* achenes of similar stage of maturity.[16]

BIDENS INVOLUCRATA retrorsa, var. nov.—A specie differt: achaeniorum aristis retrorsum hamosis.

*B. F. Bush* 5175 *pro parte*, Webb City, Missouri, September 25, 1908 (Herb. Gray, type; *non in* Herb. N.Y. Bot. Gard., *quo idem* 5175 *B. aristosa est*).

Several American species of *Bidens* have been shown to have a more or less definitely pronounced form with achenial awns barbed in the opposite way to that for those of the typical form. Thus we have *B. frondosa* var. *anomala* Port. *ex* Fern., *B. aristosa* var. *Fritcheyi* Fern., and *B. heterodoxa* var. *orthodoxa* Fern. With a very few species of *Bidens*, such as *B. ambigua* S. Moore of Africa, the direction of the barbs on the aristae appears to vary somewhat indiscriminately. For the species of the United States and Canada the direction appears more definitely fixed, however, and its reversal, noted in certain often widely scattered localities, may afford, as held by FERNALD,[17] the basis for legitimate varietal segregation. In accordance with FERNALD's view, the rare form of *B. involucrata* collected by BUSH is named var. *retrorsa*.[18]

BIDENS FRONDOSA var. pallida (Wieg.), comb. nov.; *B. melano-carpa* var. *pallida* Wieg., Bull. Torr. Bot. Club **26**:406. 1899.

When WIEGAND's *Bidens melanocarpa* was shown by E. L. GREENE (Pittonia 4:246–250. 1901) to be identical with and referable to *B. frondosa* L., the var. *pallida* was overlooked. I have had no opportunity of repeating WIEGAND's field studies of this interesting form, and so rely upon his judgment as to its varietal status. In the scanty herbarium material examined by me, however, it had seemed that extended field observations might indicate a somewhat lower than varietal rank as ordinarily interpreted.

BIDENS MICRANTHA var. laciniata (Hillebr.), comb. nov.; *Campylotheca micrantha* var. *laciniata* Hillebr. Fl. Hawaiian Isls. 216.

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[16] Dr. S. F. BLAKE, of the U.S. Bureau of Plant Industry, has kindly examined the type and likewise concluded that the plant was closer to *Bidens* than to *Coreopsis*.

[17] See also Wiegand, Bull. Torr. Bot. Club 26:400–401. 1899.

[18] For those who refuse to abide by the unfortunate provision, Article 50, of the Vienna Code (permitting a name to stand when there exists "an earlier homonym which is universally regarded as invalid"), the name becomes *Bidens polylepis* var. *retrorsa* (*cf.* Sherff, BOT. GAZ. 76:160. 1923).

1888.—A form fairly well marked but not enough to merit separate specific rank.

The earlier binomial *Bidens micrantha* Gaud. is used here since the genus *Campylotheca*, as I have previously stated (Bot. Gaz. **70**:91–96. 1920), does not appear to me worthy of retention.

BIDENS PILOSA var. **minor** (Bl.), comb. nov.; *Kerneria dubia* Cass., Dict. Sc. Nat. **24**:398. 1822 (*pro parte*); *Bidens sundaica* Blume *et* var. *minor* Blume Bijdr. 913. 1826; *B. leucantha* var. *sundaica* (Bl.) Hasskarl, Cat. Pl. Hort. Bog. 100. 1844; *etiam in* Miquel Fl. Ned. **2**:77. 1856–1859; *B. aurantiaca* Colenso, Trans. New Zeal. Inst. **27**:388. 1895; *B. pilosa* var. *dubia* (Cass.) O. E. Schulz, Urban Symb. Antill. **7**:135. 1911.

*Bidens pilosa* L. has among its various varieties and forms a subradiate variety, widely distributed in both hemispheres. It apparently was first observed by CASSINI.[19] O. E. SCHULZ (*l.c.*) has employed for it the varietal name *dubia*, but the synonymous *Bidens sundaica* Bl. had already been reduced to varietal rank by HASSKARL (*l.c.*) in 1844, and so takes precedence over the name *dubia*. In his original description, however, BLUME (*l.c.*) listed a variety *minor*, "caule foliisque humilioribus. Crescit: cum praecedente [i.e., *B. sundaica ips.*, prope Buitenzorg, Java]." Recently, through the great kindness of Dr. J. W. C. GOETHART, Director of the Rijks Herbarium at Leyden, I was privileged to examine early specimens by BLUME and by JUNGHUHN, from Java. Three sheets (nos. 900,146 . . . . 72 [*pro parte*]; 900,146 . . . . 73; 900,146 . . . . 75) bear excellent specimens of BLUME's *Bidens sundaica*. A few minute rays are present. The first of these sheets bears also the basal part of a specimen (and, in addition, a fruiting branch which probably belonged to it), labeled unmistakably in BLUME's own handwriting, "*Bidens Sundaica Variet.*" Dr. GOETHART (*in litt.*) very properly regards this as "probably type of var. *minor*." The one fruiting head still attached to the rather depauperate basal portion exactly matches the others on the *B. sundaica* plants. A fourth sheet bears still more depauperate material collected by Dr. FR. JUNGHUHN, no. 357, on Mt. Dieng, Java. It is labeled in MIQUEL's[20] handwriting (*fide* Goethart *in litt.*) as a depauperate *forma* of *B. sun-*

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[19] ". . . . les calathides, composées d'un disque jaune et d'une couronne blanchâtre, sont larges de cinq lignes. . . . . Nous avons observé, pendant plusiers années, des individus vivans de cette espèce, cultivés au Jardin du Roi, et nous avons remarqué que leurs calathides etoient le plus souvent incouronnées, rarement radiées. Dans ce dernier cas, la couronne étoit composée de cinq à sept fleurs, dont la corolle avoit le tube court, et la languette courte, large, orbiculaire, tridentée au sommet, multinervée, à nervures jaunâtres . . . .," CASSINI, *l.c.*

[20] MIQUEL (*l.c.*) cites a depauperate form collected by JUNGHUHN: "*B. leucantha β sundaica* Hassk. *l.c.* p. 100, cujus auctoritate formam hanc alioquin satis constantem huc retuli.—Formam omnibus partibus depauperatam in elevationibus montanis Javae insulae cl. Jungh. legit."

*daica* Bl. Indeed, its three specimens (one of them, in flower, having the tiny rays very evident) are so obviously a merely less developed form of *B. sundaica*, that few if any authors today would seek to draw any distinctions.

It is evident, then, that the actually equivalent name *minor*, given varietal status long before the name *sundaica*, must in turn take precedence, supplanting both *sundaica* and *dubia*.[21]

BLUME's description, "B. floribus subradiatis . . . ." is matched by upward of a thousand specimens examined by me in the past few years.[22] It is represented in particular by several of BLUME's own specimens in Herb. Brit. Mus. (where numbered 139), Herb. DC. Prodr. (*in* Herb. Deless.), Herb. Par., and Herb. Webb. In the last three herbaria the heads are mostly rayless, perhaps having had the few rays removed in previous examinations. The foliage aspect, however, is definitely that of the ordinary subradiate variety. Furthermore, the plant at the British Museum (of Natural History) is definitely subligulate.[23]

*Bidens aurantiaca* Colenso has its description ("Florets . . . . small, bright dark orange: ray—few, . . . .") supplemented by the authentic *Colenso* specimen at Kew. This has the one flowering head now lacking rays, but the aspect of the various critical structures is more properly that of the variety *minor* than of *B. pilosa* proper.


## EXPLANATION OF PLATES XIX–XXII

### PLATE XIX

*Coreopsis ochracea; a, b,* flowering branch and foliage, ×0.63; *c, d,* exterior involucral bracts, ×2.5; *e,* interior involucral bract, ×2.5; *f,* ligulate floret, ×1.9; *g,* palea, ×2.5; *h,* disc floret, ×2.5; *i,* immature achene, ×4; all from *W. Goetze* 731, type, in Herb. Berl.

### PLATE XX

*Bidens pilosa* var. *calcicola:* a, flowering and fruiting specimen, ×0.64; *b,* exterior involucral bract, ×4.5; *c,* interior involucral bract, ×4.5; *d,* ligulate floret, ×4.5; *e,* palea, ×4.5; *f,* disc floret, ×4.5; *g,* outer and *h,* inner achenes, ×4.5; *a* from *Kerber,* Cordoba, Vera Cruz, Mexico, July 22, 1882, in Herb. Berl.; *b–h,* from *Berlandier* 5 and 113, cotypes in Herb. Par.

[21] *Cf.* O. E. SCHULZ (Engler Bot. Jahrb. 50 [Suppl.] 179. 1914), " . . . . dagegen gehören *B. sundaicus* . . . . und var. *minor* . . . . wahrscheinlich zu *B. pilosus* L. var. *dubius* (Cass.) O. E. S."

[22] My manuscript lists more than 150 collections, but many others had been omitted for lack of space.

[23] In my own cultural observations, the var. *minor* appeared to come very true to a given type. Thus, for example, in some two dozen plants (*Sherff* 2043, Herb. Field Mus., etc.), raised in 1915 from achenes on specimens sent me by M. ST. AHNNE (Bot. Gard. Raoul, Papeete, Isl. Tahiti), the hundreds of flowering heads had uniformly tiny, distinctly yellowish ligulate florets, as had been observed on the parent material.

C. E. SHERFF DEL.

PLATE XX



E. E. SHERFF DEL.

E. E. SHERFF DEL.

E. E. SHERFF DEL.
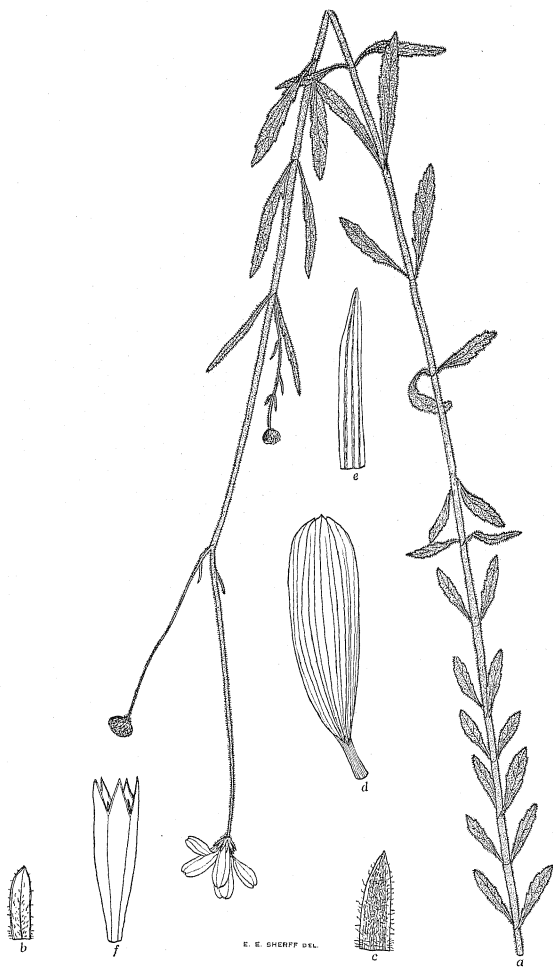
SHERFF on COMPOSITAE
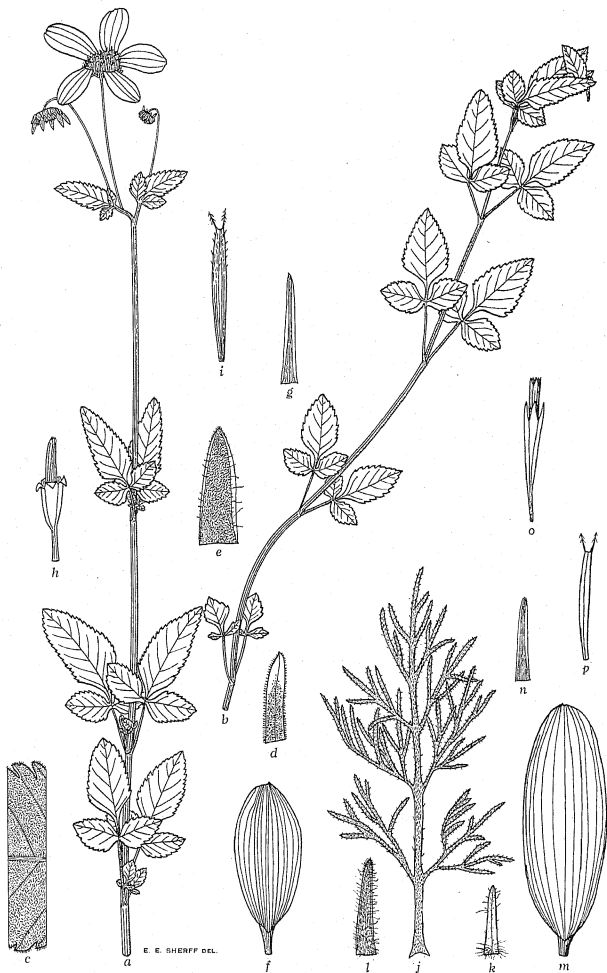
*PLATE XXI*

*Bidens triplinervia* (atypic, the form described for *B. hirtella*): *a*, flowering branch, ×0.72; *b*, exterior involucral bract, ×0.86; *c*, interior involucral bract, ×0.86; *d*, ligule, ×2.9; *e*, palea, ×0.86; *f*, corolla of disc floret, ×0.86; all from Humboldt and Bonpland, type of *B. hirtella*, in Herb. Par.

*PLATE XXII*

*Bidens triplinervia* var. *mollis*: *a*, *b*, flowering and foliage branches, ×0.73; *c*, portion of leaf, ×2.9; *d*, exterior involucral bract, ×4.4; *e*, interior involucral bract, ×4.4; *f*, ligule, ×2.2; *g*, palea, ×4.4; *h*, corolla of disc floret, ×4.4; *i*, achene, ×4.4; *a*, *b*, from *R. Spruce* 5047, at foot of Mt. Tunguragua, Ecuador, Herb. Mus. Vienna; *c–h* from *E. Poeppig* 1377, type, in Herb. Mus. Vienna; *B. triplinervia* var. *macrantha*, atypic, the form described for *B. attenuata*): *j*, stem leaf, ×1.46 (seen from beneath, leaves mostly flattened out but petiole left convolute, its ciliation thus not showing) *k*, exterior involucral bract, ×4.4; *l*, interior involucral bract, ×4.4; *m*, ligule, ×2.2; *n*, palea, ×4.4; *o*, corolla of disc floret, ×4.4; *p*, achene, ×4.4; *j–p* from *Ghiesbreght* 85 *pro parte*, Chiapas, Mexico, type in Herb. Gray.

# EFFECTS OF LIME AND POTASH FERTILIZERS ON CERTAIN MUCK SOILS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 342

WALTER FERDINAND LOEHWING

(WITH SEVEN FIGURES)

## Introduction

Ordinary methods of fertilizer practice have repeatedly been found poorly adapted to muck soils. Applications of mineral nutrients, apparently deficient in these organic soils, have frequently proved injurious instead of beneficial to crops in the field. Injurious results from applications of lime and potash have been reported for mucks exhibiting positive acidity and chemical deficiency of potassium. Results of experiments on such soils are mentioned more specifically later. In this report analyses of plants grown on such muck soils are presented for comparison with those of plants grown on soils showing favorable responses to lime and potash applications, in order to disclose the difference in the balance of nutrients within the tissues which accompany injury from fertilizers.

The term muck is here employed to designate dark colored or black soils high in organic matter, light in weight, and pulverulent or downy in texture. Mucks are distinguished from peats by their greater weight and decomposition of vegetable detritus, plant structures being unrecognizable. Mucks often resemble fertile humus soils in color and texture, but differ widely from them in organic content, temperature, and water retentivity. Ability to cultivate peats and mucks would add millions of acres to the world's most valuable farm lands, since these soils lie predominantly in the grain belts. DACHNOWSKI (6) estimates that Europe has 77 million, Canada 30 million, and the United States 20 million acres of unproductive peats and mucks. The works of SOPER (25), DACHNOWSKI, BERSCH (3), SEELHORST (24), and PUCHNER (21) review the enormous literature on this subject, and bring up to date the existing information on agricultural practices adapted to these soils.

Fertilizer tests showing injurious effects of lime on corn in swamp lands of Kankakee, Mason, and Tazewell Counties, Illinois, have been reported by HOPKINS, READHIMER, AND FISHER (12). These were four year field experiments in which the effects of limestone were consistently injurious to corn. GAMBLE and SLATER (10) reported a five year field test in which the effects of lime and potassium on oats proved beneficial when applied separately, but injurious when combined. Fertilizer tests on muck soils of Indiana reported by CONNER and ABBOTT (5) show some soils which do not respond favorably to any ordinary treatment. CONNER also reported a muck from Newton County, Indiana, showing a lime requirement of 20,000 parts per million which was not benefited by lime applications. European investigators have also reported diminution in crop yields following fertilizer applications on muck soils. Thus EHRENBERG (8, 9), ARND (1), and DUMONT and CROCHETELLE (7) have pointed out that lime and potash fertilizers may often prove deleterious, even though soil analyses reveal apparent deficiencies in these elements. The mucks studied by these investigators, as well as those discussed in this report, do not include "alkali" or "bogus" soils excessively high in soluble salts, iron, or aluminum. CONNER (4) shows that unproductive black soils containing excess soluble salts differ in numerous ways from true mucks.

## Experimental procedure

Mucks known to show a decided response to lime and potash in the field were collected at various localities and shipped to the University of Chicago. Seven pounds of each soil were placed in glazed, one gallon earthenware pots, and treated with mineral fertilizers as indicated later. Each series was set up in duplicate for purposes of comparison and to insure ample material for analysis. Tests were conducted in a greenhouse with the temperature maintained between 18° and 27° C. After planting, water was added to soils until the weight of pot contents was 8.5 pounds, the water representing approximately 20 per cent of the soil weight. This apparently optimum moisture content was readily maintained throughout the experiments, as evaporation in the greenhouse was only moderate. Four different crop plants were tested, including Marquis Hard

wheat, Yellow Dent corn, Victory oats, and Mammouth Red Fancy
clover. Wheat, oats, and clover were thinned to fifteen and corn to
five plants per pot at the end of the second week. Each crop was
grown on four muck soils which differed in their responses to potash
and lime amendments. At the end of the tenth week, pot contents
were removed *in totem* to a pan, roots washed free of soil, rinsed in
distilled water, and dried on filter paper. The entire crop from each
pot was then weighed and hashed in a Nixtamel mill. After thorough
mixing, samples were taken for moisture determinations, and the
remaining green hash pickled for storage in 80 per cent boiling alco-
hol containing 2 gm. chemically pure calcium carbonate per liter.

TABLE I

ANALYSES OF UNTREATED MUCK SOILS

|  | Soil 1 percentage | Soil 2 percentage | Soil 3 percentage | Soil 4 percentage |
|---|---|---|---|---|
| Hygroscopic moisture................... | 8.20 | 12.21 | 3.43 | 15.30 |
| Total potassium....................... | 0.29 | 1.52 | 0.30 | 1.05 |
| Total calcium......................... | 3.44 | 0.08 | 1.67 | 3.54 |
| Total magnesium...................... | 0.40 | 0.10 | 0.18 | 1.02 |
| Total iron............................ | 1.21 | 2.02 | 0.10 | 2.22 |
| Total phosphorus...................... | 0.19 | 0.08 | 0.34 | 0.20 |
| Total sulphur......................... | 0.54 | 0.11 | 0.44 | 0.20 |
| Total nitrogen........................ | 3.62 | 1.42 | 3.90 | 1.22 |
| Total volatile matter................... | 48.10 | 42.61 | 83.00 | 47.78 |
| Insoluble silicates, etc................. | 15.50 | 26.80 | 5.10 | 24.40 |
| Lime requirements in ppm.............. | 2700.00 | 3500.00 | 2700.00 | 1200.00 |

Alcohol was evaporated at the beginning of analyses, materials
transferred to glass stoppered weighing bottles, and dried to con-
stant weight in a vacuum oven at 80° C. Four soils were employed
in the experiment, as follows:

1. Black powdery acid muck from Manito, Mason County,
Illinois. This soil showed injury to cereals when lime was added to
correct acidity. It responded favorably to potash.

2. Sandy acid muck from Mill Creek, between Mud and Fish
Lakes, La Porte County, Indiana. Crops on this soil were injured
by lime and by potash.

3. Light weight acid muck from Newland, Jasper County, Indi-
ana, known to respond favorably to lime but unfavorably to potash.

4. Black acid muck from farm of Mr. PETER LARSEN, near

Hopkins, Minnesota, known to respond very favorably to lime and potash fertilizers. Analyses of these soils are given in table I.

Pots of each soil were treated with powdered, chemically pure salts as follows: Pot A (control): untreated soil; Pot B: 400 ppm

TABLE II

ANALYSES OF YELLOW DENT CORN GROWN ON FOUR MUCK SOILS

| FERTILIZER ADDED (PPM) | PLANT WEIGHT (GM.) | PERCENTAGE | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | MOIS-TURE | POTAS-SIUM | CAL-CIUM | ORGANIC NITRO-GEN | NITRATE NITRO-GEN | TOTAL SUGARS | CARBO-HYDRATE |
| | | | | Soil 1 | | | | |
| None (check) | 7.45 | 82.4 | 0.42 | 0.18 | 1.91 | 0.14 | 5.02 | 9.12 |
| 400 KCl | 8.50 | 79.8 | 0.78 | 0.14 | 2.05 | 0.04 | 7.12 | 11.13 |
| 4000 CaCO₃ | 6.20 | 82.7 | 0.30 | 0.21 | 1.65 | 0.09 | 4.54 | 8.04 |
| 4000 CaCO₃ 400 KCl | 7.10 | 81.7 | 0.74 | 0.22 | 1.74 | 0.20 | 4.87 | 8.98 |
| 8000 CuCO₃ 400 KCl | 7.15 | 82.4 | 0.60 | 0.22 | 1.70 | 0.10 | 4.66 | 8.01 |
| | | | | Soil 2 | | | | |
| None (check) | 6.88 | 81.4 | 0.45 | 0.15 | 1.82 | 0.12 | 6.20 | 10.11 |
| 400 KCl | 4.75 | 82.8 | 0.62 | 0.14 | 1.75 | 0.18 | 4.40 | 9.10 |
| 4000 CaCO₃ | 5.05 | 82.1 | 0.42 | 0.23 | 1.54 | 0.35 | 6.09 | 8.42 |
| 4000 CaCO₃ 400 KCl | 5.20 | 80.9 | 0.43 | 0.23 | 1.50 | 0.28 | 6.17 | 8.98 |
| 8000 CaCO₃ 400 KCl | 5.40 | 80.7 | 0.44 | 0.23 | 1.71 | 0.09 | 5.85 | 8.90 |
| | | | | Soil 3 | | | | |
| None (check) | 8.40 | 82.7 | 0.62 | 0.13 | 1.85 | 0.08 | 3.67 | 10.59 |
| 400 KCl | 8.00 | 81.7 | 0.65 | 0.10 | 1.80 | 0.40 | 5.36 | 9.67 |
| 4000 CaCO₃ | 5.80 | 81.0 | 0.58 | 0.20 | 1.58 | 0.28 | 3.81 | 8.28 |
| 4000 CaCO₃ 400 KCl | 7.20 | 83.4 | 0.59 | 0.21 | 1.58 | 0.31 | 3.70 | 9.50 |
| 8000 CaCO₃ 400 KCl | 7.05 | 83.6 | 0.59 | 0.29 | 1.82 | 0.11 | 3.70 | 9.59 |
| | | | | Soil 4 | | | | |
| None (check) | 4.45 | 81.1 | 0.61 | 0.11 | 1.81 | 0.36 | 5.11 | 8.41 |
| 400 KCl | 6.28 | 79.5 | 0.77 | 0.10 | 2.00 | 0.15 | 5.25 | 8.87 |
| 4000 CaCO₃ | 6.25 | 80.4 | 0.60 | 0.24 | 1.78 | 0.20 | 4.87 | 8.00 |
| 4000 CaCO₃ 400 KCl | 7.70 | 80.6 | 0.78 | 0.24 | 2.01 | 0.07 | 5.38 | 8.49 |
| 8000 CaCO₃ 400 KCl | 7.00 | 80.1 | 0.79 | 0.24 | 2.01 | 0.07 | 5.19 | 8.79 |

potassium chloride; Pot C: 4000 ppm calcium carbonate; Pot D: 4000 ppm calcium carbonate and 400 ppm potassium chloride; Pot E: 8000 ppm calcium carbonate and 400 ppm potassium chloride.

The minerals were sifted into and thoroughly mixed with the soil. Series were run in duplicate. A third series, in which calcium acid phosphate was added throughout at the rate of 400 ppm, showed that plants on these soils did not respond to phosphorus. Etiolation

effects indicative of phosphorus shortage were not noticed on any untreated soil employed in the experiments.

## Analytical methods

Soil analyses were made by the methods of the Association of Official Agricultural Chemists (2), potassium being determined as

### TABLE III

ANALYSES OF MARQUIS HARD WHEAT GROWN ON FOUR MUCK SOILS

| FERTILIZER ADDED (PPM) | PLANT WEIGHT (GM.) | PERCENTAGE | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | MOISTURE | POTASSIUM | CALCIUM | ORGANIC NITROGEN | NITRATE NITROGEN | TOTAL SUGARS | CARBOHYDRATE |
| Soil 1 | | | | | | | | |
| None (check) | 4.74 | 86.4 | 0.55 | 0.21 | 0.88 | 0.03 | 6.35 | 10.24 |
| 400 KCl | 5.80 | 88.5 | 0.78 | 0.17 | 0.90 | 0.01 | 5.77 | 12.75 |
| 4000 CaCO₃ | 2.70 | 89.0 | 0.49 | 0.28 | 0.80 | 0.73 | 3.72 | 9.14 |
| 4000 CaCO₃ 400 KCl | 3.55 | 85.2 | 0.62 | 0.10 | 1.14 | 0.10 | 4.23 | 9.01 |
| 8000 CaCO₃ 400 KCl | 3.20 | 88.1 | 0.51 | 0.27 | 0.89 | 0.51 | 3.70 | 9.24 |
| Soil 2 | | | | | | | | |
| None (check) | 4.64 | 84.2 | 0.61 | 0.22 | 0.92 | 0.72 | 4.77 | 11.45 |
| 400 KCl | 2.30 | 86.4 | 0.77 | 0.18 | 0.78 | 0.42 | 3.32 | 8.07 |
| 4000 CaCO₃ | 3.81 | 85.3 | 0.57 | 0.41 | 0.92 | 0.76 | 4.25 | 9.44 |
| 4000 CaCO₃ 400 KCl | 3.28 | 87.0 | 0.65 | 0.20 | 0.92 | 0.26 | 4.00 | 9.06 |
| 8000 CaCO₃ 400 KCl | 2.95 | 87.7 | 0.50 | 0.44 | 0.87 | 0.91 | 4.29 | 9.54 |
| Soil 3 | | | | | | | | |
| None (check) | 5.52 | 82.2 | 0.31 | 0.31 | 1.71 | 0.00 | 5.21 | 10.70 |
| 400 KCl | 4.32 | 85.4 | 0.61 | 0.19 | 1.22 | 0.59 | 5.46 | 8.92 |
| 4000 CaCO₃ | 2.66 | 82.7 | 0.21 | 0.42 | 0.68 | 0.82 | 3.21 | 9.70 |
| 4000 CaCO₃ 400 KCl | 3.15 | 83.9 | 0.25 | 0.34 | 0.90 | 0.78 | 5.20 | 8.42 |
| 8000 CaCO₃ 400 KCl | 2.98 | 82.3 | 0.24 | 0.34 | 0.90 | 0.69 | 4.94 | 8.66 |
| Soil 4 | | | | | | | | |
| None (check) | 3.97 | 83.0 | 0.61 | 0.18 | 0.73 | 0.69 | 3.92 | 9.02 |
| 400 KCl | 3.82 | 84.6 | 0.70 | 0.10 | 0.97 | 0.47 | 4.83 | 10.19 |
| 4000 CaCO₃ | 4.47 | 82.7 | 0.44 | 0.31 | 0.99 | 0.71 | 5.22 | 10.80 |
| 4000 CaCO₃ 400 KCl | 4.93 | 82.3 | 0.76 | 0.34 | 1.03 | 0.74 | 5.61 | 10.52 |
| 8000 CaCO₃ 400 KCl | 4.28 | 81.8 | 0.60 | 0.33 | 0.82 | 0.41 | 4.44 | 9.45 |

its chloroplatinate, and calcium titrated as oxalate against potassium permanganate. Lime requirement in parts per million was determined by the Veitch method (15).

Entire plants including roots were analyzed. Moisture content is expressed as percentage of wet weight. Potassium, calcium, and

phosphorus were determined by sulphuric acid combustion methods. The oxidized residues were transferred to platinum crucibles, and the sulphuric acid carefully evaporated on hot plates, followed by ignition to drive off ammonia for calcium and potassium determinations. Calcium was precipitated as oxalate, and potassium as its

TABLE IV

ANALYSES OF VICTORY OATS GROWN ON FOUR MUCK SOILS

| FERTILIZER ADDED (PPM) | PLANT WEIGHT (GM.) | PERCENTAGE | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | MOIS-TURE | POTAS-SIUM | CAL-CIUM | ORGANIC NITRO-GEN | NITRATE NITRO-GEN | TOTAL SUGARS | CARBO-HYDRATE |
| Soil 1 | | | | | | | | |
| None (check)............ | 4.38 | 81.1 | 1.01 | 0.17 | 1.81 | 0.20 | 3.83 | 8.45 |
| 400 KCl................ | 4.87 | 82.3 | 1.24 | 0.22 | 2.02 | 0.02 | 4.02 | 9.05 |
| 4000 CaCO₃............. | 2.76 | 87.0 | 0.99 | 0.25 | 1.80 | 0.80 | 4.11 | 7.60 |
| 4000 CaCO₃ 400 KCl.... | 4.05 | 84.7 | 1.23 | 0.23 | 2.07 | 0.35 | 3.95 | 8.24 |
| 8000 CaCO₃ 400 KCl.... | 3.16 | 86.9 | 1.00 | 0.25 | 1.98 | 0.58 | 4.71 | 7.78 |
| Soil 2 | | | | | | | | |
| None (check)............ | 4.24 | 81.5 | 0.77 | 0.15 | 1.40 | 0.31 | 4.46 | 8.97 |
| 400 KCl................ | 2.10 | 80.5 | 0.97 | 0.09 | 1.08 | 0.06 | 5.02 | 7.80 |
| 4000 CaCO₃............. | 3.70 | 83.1 | 0.49 | 0.20 | 0.88 | 0.87 | 4.88 | 7.77 |
| 4000 CaCO₃ 400 KCl.... | 2.98 | 82.7 | 0.75 | 0.16 | 1.18 | 0.02 | 5.41 | 8.37 |
| 8000 CaCO₃ 400 KCl.... | 2.46 | 82.4 | 0.48 | 0.20 | 1.07 | 0.84 | 5.00 | 7.60 |
| Soil 3 | | | | | | | | |
| None (check)............ | 3.46 | 84.3 | 0.44 | 0.28 | 1.74 | 0.14 | 3.34 | 10.64 |
| 400 KCl................ | 3.12 | 81.3 | 0.64 | 0.20 | 1.40 | 0.20 | 3.74 | 9.92 |
| 4000 CaCO₃............. | 2.50 | 82.0 | 0.32 | 0.40 | 1.01 | 0.56 | 3.20 | 9.25 |
| 4000 CaCO₃ 400 KCl.... | 2.69 | 84.6 | 0.36 | 0.39 | 1.23 | 0.21 | 3.82 | 9.75 |
| 8000 CaCO₃ 400 KCl.... | 2.39 | 82.4 | 0.31 | 0.39 | 1.02 | 0.24 | 3.71 | 9.03 |
| Soil 4 | | | | | | | | |
| None (check)............ | 3.11 | 84.2 | 0.68 | 0.18 | 1.63 | 0.59 | 4.47 | 8.99 |
| 400 KCl................ | 3.70 | 86.2 | 0.78 | 0.14 | 1.82 | 0.49 | 5.26 | 9.42 |
| 4000 CaCO₃............. | 3.24 | 83.4 | 0.65 | 0.19 | 1.84 | 0.36 | 5.42 | 9.32 |
| 4000 CaCO₃ 400 KCl.... | 3.82 | 84.5 | 0.76 | 0.21 | 1.88 | 0.26 | 5.56 | 9.84 |
| 8000 CaCO₃ 400 KCl.... | 3.01 | 82.5 | 0.74 | 0.19 | 1.63 | 0.22 | 5.31 | 9.79 |

cobaltinitrite. Each was titrated against standard potassium permanganate. Phosphorus was determined as ammonium phosphomolybdate by the Neumann-Pemberton (2) method, but is not reported because its variations (between 0.15 and 0.38 per cent) were small and apparently of no significance. Nitrogen was determined by the Gunning method (2). Tables II–V show as organic nitrogen

the percentage of nitrogen obtained without the use of salicylic
acid in the digestion mixture. Nitrate nitrogen represents the in-
crease over organic nitrogen in nitrogen content found when diges-
tion was performed with a one to thirty salicylic-sulphuric acid

TABLE V

ANALYSES OF MAMMOTH RED FANCY CLOVER GROWN ON
FOUR MUCK SOILS

| FERTILIZER ADDED (PPM) | PLANT WEIGHT (GM.) | PERCENTAGE | | | | | | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| | | MOIS-TURE | POTAS-SIUM | CAL-CIUM | ORGANIC NITRO-GEN | NITRATE NITRO-GEN | TOTAL SUGARS | CARBO-HYDRATE |
| | | | | Soil 1 | | | | |
| None (check) | 2.00 | 81.7 | 0.71 | 0.22 | 2.31 | 0.26 | 4.81 | 10.42 |
| 400 KCl | 1.72 | 83.5 | 0.08 | 0.18 | 1.75 | 0.43 | 4.00 | 10.07 |
| 4000 CaCO3 | 2.41 | 80.9 | 0.70 | 0.28 | 2.49 | 0.03 | 5.41 | 11.44 |
| 4000 CaCO3 400 KCl | 2.16 | 80.8 | 0.84 | 0.26 | 1.98 | 0.34 | 4.08 | 10.06 |
| 8000 CaCO3 400 KCl | 2.39 | 80.5 | 0.85 | 0.28 | 2.00 | 0.11 | 3.85 | 8.97 |
| | | | | Soil 2 | | | | |
| None (check) | 1.05 | 82.7 | 0.80 | 0.19 | 2.42 | 0.01 | 5.27 | 8.90 |
| 400 KCl | 1.46 | 83.2 | 0.99 | 0.17 | 1.82 | 0.60 | 4.81 | 7.05 |
| 4000 CaCO3 | 2.18 | 82.8 | 0.75 | 0.23 | 1.70 | 0.41 | 5.44 | 7.81 |
| 4000 CaCO3 400 KCl | 1.74 | 82.0 | 0.64 | 0.23 | 1.48 | 0.62 | 5.77 | 7.57 |
| 8000 CaCO3 400 KCl | 1.90 | 82.2 | 0.09 | 0.24 | 1.48 | 0.61 | 3.24 | 6.71 |
| | | | | Soil 3 | | | | |
| None (check) | 2.41 | 80.7 | 0.41 | 0.11 | 1.31 | 0.04 | 4.42 | 8.50 |
| 400 KCl | 1.72 | 81.6 | 0.07 | 0.10 | 1.44 | 0.08 | 4.47 | 8.67 |
| 4000 CaCO3 | 2.26 | 81.6 | 0.09 | 0.16 | 1.50 | 0.11 | 3.21 | 10.77 |
| 4000 CaCO3 400 KCl | 2.18 | 81.5 | 0.08 | 0.15 | 1.57 | 0.04 | 3.92 | 9.09 |
| 8000 CaCO3 400 KCl | 2.40 | 81.6 | 0.09 | 0.15 | 1.54 | 0.08 | 3.41 | 9.41 |
| | | | | Soil 4 | | | | |
| None (check) | 1.78 | 82.4 | 0.55 | 0.15 | 2.16 | 0.37 | 3.58 | 8.78 |
| 400 KCl | 1.25 | 81.5 | 0.84 | 0.16 | 2.54 | 0.14 | 5.41 | 10.12 |
| 4000 CaCO3 | 2.27 | 82.0 | 0.42 | 0.22 | 2.71 | 0.10 | 6.35 | 10.42 |
| 4000 CaCO3 400 KCl | 2.04 | 81.7 | 0.90 | 0.28 | 2.70 | 0.07 | 5.98 | 10.48 |
| 8000 CaCO3 400 KCl | 1.62 | 81.3 | 0.52 | 0.26 | 2.25 | 0.30 | 4.82 | 10.00 |

mixture. The quantities of nitrate nitrogen in these young plants
appeared great enough to justify determination.

Total sugars were determined in the $F_1$ and $F_2$ (water-alcohol-
ether soluble) fractions of the tissue extracts by the Bertrand volu-
metric method. Copper values are expressed in glucose equivalents
as given in the Munson-Walker tables (2). Percentages are ex-

pressed on the basis of total dry weight of the sample ($F_1$ plus $F_2$ plus $F_3$).

Total carbohydrates include the carbohydrates, calculated as starch, in the insoluble $F_3$ fraction hydrolyzed by strong hydrochloric acid, plus the total soluble sugars of the $F_1$ and $F_2$ fractions.

### Discussion

The data confirm the oft proved and well known fact that it is impossible to determine the fertilizer needs of soils from their chemical analyses. Soil 1, high in calcium and low in potassium, responded
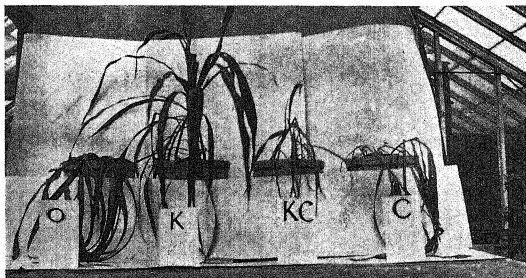


FIG. 1.—Injurious effect of calcium carbonate on Soil 1: $O$, check; $K$, 400 ppm KCl; $KC$, 400 ppm KCl plus 4000 ppm $CaCO_3$; $C$, 4000 ppm $CaCO_3$.

favorably to potash but unfavorably to lime, as shown in fig. 1. Soil 2, high in potash but low in lime, responded unfavorably to both fertilizers (fig. 2). Soil 3, high in potash and medium in lime, was injured by potassium (fig. 3), while Soil 4, high in both, responded favorably to both, as shown in fig. 4. The injurious effects of lime are astonishing in view of the known acidity of these soils, and must consequently be explained by the action of lime on soil nutrients, the net result of which is unfavorable to crops. ROBINSON (23), working on Michigan mucks, found that liming frequently was injurious, in some cases entirely offsetting the benefit of potassium fertilizers. NOLTE (17) points out that alkali salts increase soil density and capillary water capacity, making them physiologically

dry, a conclusion confirmed by EHRENBERG (9), who also has found that too much lime may depress potassium assimilation. The importance of the physiological availability of potassium and calcium salts is shown by the fact that their concentration in tissues
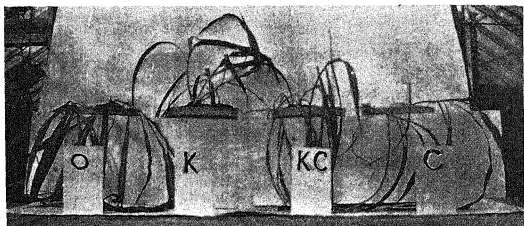


FIG. 2.—Injurious effect of potassium chloride and calcium carbonate on Soil 2: O, check; K, 400 ppm KCl; KC, 400 ppm KCl plus 4000 ppm CaCO₃; C, 4000 ppm CaCO₃.
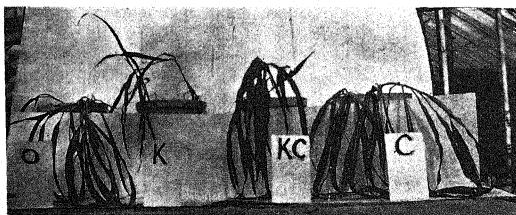


FIG. 3.—Injurious effect of potassium chloride on Soil 3: O, check; K, 300 ppm KCl; KC, 400 ppm KCl plus 4000 ppm CaCO₃; C, 4000 ppm CaCO₃.

varies independently of the concentration of these bases in the soils.

Ordinary experimental methods for determination of availability of plant foods in soils are not adapted to mucks. It was found extremely difficult to avoid the adsorptive effects of organic colloids. Soil extracts, initially acid to indicators, may turn alkaline on stand-

ing. Colloid phenomena such as these were accompanied by great variations in concentration of plant nutrients, especially potassium and calcium. PUCHNER (22) reported similar observations on extracts of humus soils in Europe. Humus colloids form complexes with calcium, iron, and magnesium which are soluble in excess ammonia, as has been pointed out by OSTWALD (18). The selection of fertilizers on the basis of soil extracts has proved insecure for mineral soils, and is less secure for mucks. The accompanying tissue
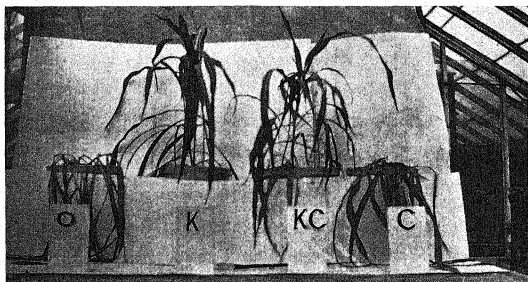


FIG. 4.—Influence of potassium fertilizer in increasing stem erectness of corn: O, check; K, 400 ppm KCl; KC, 400 ppm KCl plus 4000 ppm CaCO₃; C, 4000 ppm CaCO₃ on Soil 4.

analyses reveal some of the changes in nutrients within the plants following applications of lime and potash.

One of the most striking results shown by the experiments is the effect of potash fertilizers. Cereals treated with potash were much more erect than those untreated; the latter were decumbent and sprawling (fig. 4). KRAUS (13) and WALSTER (27) observed similar results in other crops in the absence of potassium.

The concentration of potassium and calcium increased in tissues when these salts were used as fertilizers, thus indicating increased availability in spite of the tendency of the humus colloids to adsorb mineral bases. The effect of potash on stem erectness is especially striking in view of the fact that increase in potassium induces a

depression in calcium content of cereals (tables II–IV). Abundance
of calcium alone did not seem sufficient for production of sturdy,
erect plants, since limed pots with soils low in potash usually con-
tained sprawling or lopped-over crops. Depression of calcium con-
tent by potassium was found generally to be the case except in
clover (table V).

Calcium likewise diminished the potash content of tissues in all
cases, including clover, showing the balance between lime and pot-
ash to be a very delicate one. These observations coincide with
those of EHRENBERG (9), who used lime and potash fertilizers for
wheat and barley grown in sand cultures. From his own results and
the data of many other workers, which are reviewed by him, EHREN-
BERG derived his well known lime-potash law. He states that, when
lime intake is greatly increased in plants poorly supplied with po-
tassium, absorption of the latter is diminished, often making its
absence a limiting factor in development. EHRENBERG'S analyses do
not give calcium, thus leaving the effect of potassium on calcium
content of tissues in his experiments unknown. By the use of data
given in tables II–V, it is possible to express the effects of potassium
and calcium fertilizers on the calcium and potassium content of
tissues as a ratio of one to the other, the value of this ratio, $K/Ca$ in
the tissues, being diminished by lime but increased by potash ferti-
lizers. These changes are usually caused by an actual depression
of one element and a positive increase of the other in plants. In
clover the best crop yields were uniformly associated with a low
$K/Ca$ ratio; this was not the case in cereals. From these facts it
appears that the value of the $K/Ca$ ratio is more significant in con-
nection with crop production of clover than of cereals. This may
hold true for the legumes generally. The better grain crops were
characterized by relatively high potassium and nitrogen content,
rather than by any particular value of the $K/Ca$ ratio.

From the data in table VI it is apparent that potash uncombined
with lime may prove undesirable as a muck fertilizer for clover.
VANDECAVEYE (26), working with potassium fertilizers, found them
beneficial to wheat but not to clover. Only on Soil 4 did potash
seem beneficial for clover, and even in this case not beneficial per se,
but rather because potash assisted in increasing the calcium content

of the tissues. As there were no marked differences in root systems of the cultures, it appears that the injurious action of potassium on clover is due to disturbance of normal carbohydrate and protein synthesis. Dry weight, soluble sugars, total carbohydrates, and organic nitrogen of clover increased with rise in calcium content but diminished with increase in K/Ca ratio. These nutrient relations did not occur uniformly in cereal crops, and possibly they may represent distinctive differences between leguminous and non-leguminous types of plants.

Injurious effects of a given fertilizer seem to predominate when it

TABLE VI

RELATION OF K/CA RATIO TO CROP YIELD IN CLOVER

| SOIL NO. | TREATMENT (PPM) | AVERAGE WEIGHT (GM.) PER PLANT | POTASSIUM PERCENTAGE | CALCIUM PERCENTAGE | K/CA RATIO |
|---|---|---|---|---|---|
| 1B............. | 400 KCl | 1.72 | 0.98 | 0.18 | 5.4 |
| 1C............. | 4000 CaCO₃ | 2.41 | 0.70 | 0.28 | 2.6 |
| 2B............. | 400 KCl | 1.46 | 0.99 | 0.17 | 5.8 |
| 2C............. | 4000 CaCO₃ | 2.18 | 0.75 | 0.23 | 3.2 |
| 3B............. | 400 KCl | 1.72 | 0.67 | 0.10 | 6.7 |
| 3C............. | 4000 CaCO₃ | 2.46 | 0.69 | 0.16 | 4.3 |
| 4B............. | 400 KCl | 1.25 | 0.84 | 0.16 | 5.3 |
| 4C............. | 4000 CaCO₃ | 2.27 | 0.42 | .022 | 1.5 |

is applied in a mixture with another known to be beneficial. Injury is marked externally by retardation of growth, etiolation and lopping-over of plants. On Soil 1, showing injury to grains following lime applications, the injurious effect of lime predominated when both lime and potash were applied, although potash alone induced a favorable response on this soil, as shown in fig. 1. This behavior is similar to one reported by ROBINSON (23), working with beets on mucks in Michigan. According to EHRENBERG'S (9) interpretation of the action of lime, addition of potassium should have offset injury due to lime. The fact that this was not the case may be due to the high absorptive capacity of mucks. On Soil 3 lime singly was beneficial, but potassium chloride singly proved injurious, its injurious effect prevailing when it and calcium carbonate were ap-

plied together. It is questionable, of course, whether the injury here
was due to potassium or chloride ions, as mucks may adsorb po-
tassium, leaving harmful chlorides in the soil. Such preferential
adsorption may explain the responses on Soil 3 and on Soil 2, for
which neither lime nor potash proved beneficial. In short, in the
experiments here reported, the effects of the injurious fertilizer of a
combination seemed to predominate over those of the beneficial

TABLE VII

EFFECTS OF INJURIOUS FERTILIZERS ON NITROGEN AND
CARBOHYDRATE CONTENT OF TISSUES

| SOIL NO. | TREATMENT (PPM) | CROP | WEIGHT PER PLANT IN GRAMS | INJURED BY | ORGANIC NITROGEN PERCENTAGE | TOTAL CARBOHYDRATES PERCENTAGE |
|---|---|---|---|---|---|---|
| 1A.......... | None (check) |  | 8.24 |  | 1.91 | 9.12 |
| 1C.......... | 4000 CaCO₃ | Corn | 6.20 | Ca | 1.65 | 8.04 |
| 3A.......... | None (check) |  | 8.40 |  | 1.85 | 10.59 |
| 3B.......... | 400 KCl |  | 8.00 | K | 1.80 | 9.67 |
| 1A.......... | None (check) |  | 4.74 |  | 0.88 | 10.24 |
| 1C.......... | 4000 CaCO₃ | Wheat | 2.70 | Ca | 0.80 | 9.14 |
| 3A.......... | None (check) |  | 5.52 |  | 1.71 | 10.70 |
| 3B.......... | 400 KCl |  | 4.32 | K | 1.22 | 8.92 |
| 1A.......... | None (check) |  | 4.38 |  | 1.81 | 8.45 |
| 1C.......... | 4000 CaCO₃ | Oats | 2.76 | Ca | 1.80 | 7.60 |
| 3A.......... | None (check) |  | 3.46 |  | 1.74 | 10.64 |
| 3B.......... | 400 KCl |  | 3.12 | K | 1.40 | 9.92 |
| 1A.......... | None (check) |  | 2.00 |  | 2.31 | 10.42 |
| 1B.......... | 400 KCl | Clover | 1.72 | K | 1.75 | 10.07 |
| 2A.......... | None (check) |  | 1.95 |  | 2.42 | 8.90 |
| 2B.......... | 400 KCl |  | 1.46 | K | 1.82 | 7.05 |

much as though the latter fertilizer were not present. These results
point to an absence of ionic antagonism, as reported by LOEW (14),
GERICKE (11), and others, in which one ion entirely counteracted the
deleterious effects of another. Injury from fertilizers is characterized
internally by a depression of the organic nitrogen and total carbo-
hydrate content of the tissues, as shown in table VII and fig. 5.

   High calcium tissues of the cereals contained a relatively high
amount of nitrate nitrogen, as shown in fig. 6 and table VIII. This
may be due to the fact that lime increases soil nitrates, as has been
reported by PIPER (19) and other investigators. Conversely, plants
having a high K/Ca ratio contained relatively smaller amounts of

nitrate nitrogen but more organic nitrogen, as shown in fig. 7, indicating that potassium may function in the synthesis of proteins as well as carbohydrates. Since high calcium signifies also low potassium content, it might be concluded that the plant cannot synthesize the abundant soluble nitrogen without the presence of more potassium.

The ratio of nitrogen to potassium, N/K in the tissues, shows the
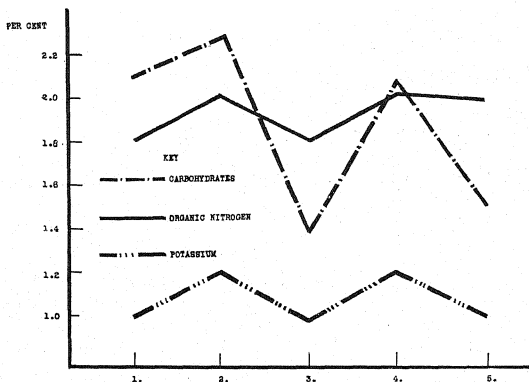


FIG. 5.—Relation of potassium to carbohydrate and organic nitrogen content of oat tissues (data from table IV, Soil I); carbohydrate percentages have been divided by 4.

balance between this fertilizer and protein synthesis, since organic nitrogen is a direct index to protein content of tissues. This N/K ratio is widened by lime primarily because the latter greatly diminishes the potassium content of plant tissues. Where this depression is too great, lack of potassium may become a limiting factor in carbohydrate and protein synthesis, resulting finally in cessation of plant growth. Lack of potassium would certainly have this effect if protein synthesis within the plant occurs in the manner that the work of PRIANISCHNIKOW (20) seems to indicate. He finds that proteins are formed from inorganic nitrogen only in the presence of

carbohydrates. He considers the stages in protein synthesis as follows: Carbohydrates+inorganic nitrogen⇌acid amides⇌amino acids⇌proteins. In the absence of carbohydrates, he finds that this process reverses, giving an accumulation of inorganic nitrogen in the plant. If potassium is essential for carbohydrate synthesis, another step is merely added to the process. It has long been known that potassium starvation interferes with carbohydrate storage,
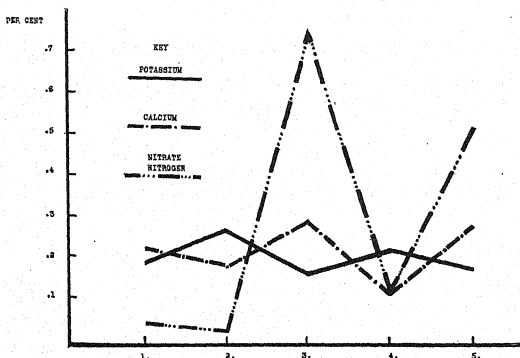


FIG. 6.—Relation of calcium and potassium to nitrate nitrogen in wheat tissues (data from table III, Soil I); potassium percentages have been divided by 3.

either by inhibiting synthesis or translocation of sugars. Carbon dioxide plus water plus potassium→carbohydrates, which, in the presence of inorganic nitrogen, lead to protein formation, as already indicated. Proteins and carbohydrates are largely substituted hydrocarbon chains in which the synthetic mechanism may not unlikely be similar, the end result depending upon the radicals replacing the original hydroxyl groups.

The analytical data reveal the fact that it is not possible to correlate crop production with either the potassium-calcium ratio or the carbohydrate-nitrogen ratio of cereal tissues, as high and low ratios among good and poor crops are almost equally common. This

may be true only in tissues of essentially young plants, such as were analyzed in this case. Mature tissues probably exhibit quite different relations, especially in connection with soluble constituents. Whether nutrient ratios in mature cereals grown on muck soils would afford an index to the character of crops remains a matter for future determination. Soluble sugars and nitrate nitrogen did not vary uniformly with insoluble carbohydrates or organic nitrogen of tissues. High yield and quality of crops seems to be correlated with high organic nitrogen and high total carbohydrates.
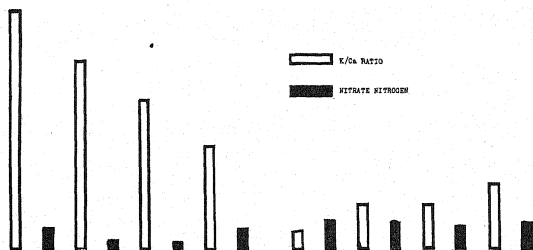


FIG. 7.—Relation of K/Ca ratio to nitrate nitrogen content of wheat tissues grown on four different soils; nitrate nitrogen content varies inversely with value of K/Ca ratio (cf. fig. 6); data from table VIII.

The relation between chemical and physical factors involved in crop production seems to be more complex on muck than on mineral soils, due largely to the greater amount of colloidal organic material in the former. As these highly colloidal soils have a great adsorption capacity, they may on one hand conserve nutrient elements by prevention of leaching, but on the other hand create a physiological deficiency of nutrients by withholding them from plants. Since bases are to some extent replaceable, the effect of a basic fertilizer on mucks might depend largely on the influence of other ions liberated by it, as well as on its own direct action. In colloids highly laden with potassium, injury to crops after heavy liming may be due to liberation of alkali carbonates and their toxic or plasmolytic effects. It is possible that lime used to correct acidity liberates such

alkalis not only by replacement, but also by its coagulative action, lime being known to coagulate colloids and reduce indices of swelling in soils (18). Alkali carbonates so liberated may prove more objec-

TABLE VIII

RELATION OF NITRATE NITROGEN TO CALCIUM AND POTASSIUM CONTENT
OF TISSUES OF CORN, WHEAT, AND OATS

| SOIL NO. | TREATMENT (PPM) | CROP | AVERAGE WEIGHT PER PLANT (GM.) | POTASSIUM PERCENTAGE | CALCIUM PERCENTAGE | K/CA RATIO | NITRATE NITROGEN PERCENTAGE |
|---|---|---|---|---|---|---|---|
| 1B...... | 400  KCl | Corn | 8.50 | 0.78 | 0.14 | 4.8 | 0.04 |
| 1C...... | 4000 CaCO₃ | Corn | 6.20 | 0.30 | 0.21 | 1.5 | 0.09 |
| 2B...... | 400  KCl | Corn | 4.75 | 0.62 | 0.14 | 4.5 | 0.18 |
| 2C...... | 4000 CaCO₃ | Corn | 5.95 | 0.44 | 0.23 | 1.8 | 0.35 |
| 3B...... | 400  KCl | Corn | 8.00 | 0.65 | 0.10 | 6.2 | 0.40 |
| 3C...... | 4000 CaCO₃ | Corn | 6.80 | 0.58 | 0.20 | 2.7 | 0.27 |
| 4B...... | 400  KCl | Corn | 6.28 | 0.77 | 0.10 | 7.6 | 0.15 |
| 4C...... | 4000 CaCO₃ | Corn | 6.25 | 0.60 | 0.24 | 2.3 | 0.20 |
| 1B...... | 400  KCl | Wheat | 5.80 | 0.78 | 0.17 | 4.4 | 0.01 |
| 1C...... | 4000 CaCO₃ | Wheat | 2.70 | 0.49 | 0.28 | 1.8 | 0.73 |
| 2B...... | 400  KCl | Wheat | 2.30 | 0.77 | 0.18 | 4.2 | 0.42 |
| 2C...... | 4000 CaCO₃ | Wheat | 3.81 | 0.57 | 0.41 | 1.2 | 0.76 |
| 3B...... | 400  KCl | Wheat | 4.32 | 0.61 | 0.19 | 3.1 | 0.59 |
| 3C...... | 4000 CaCO₃ | Wheat | 2.66 | 0.21 | 0.42 | 0.5 | 0.82 |
| 4B...... | 400  KCl | Wheat | 3.82 | 0.70 | 0.10 | 7.0 | 0.47 |
| 4C...... | 4000 CaCO₃ | Wheat | 4.47 | 0.31 | 0.99 | 1.3 | 0.71 |
| 1B...... | 400  KC. | Oats | 4.87 | 1.24 | 0.22 | 5.8 | 0.02 |
| 1C...... | 4000 CaCO₃ | Oats | 2.76 | 0.99 | 0.25 | 4.0 | 0.80 |
| 2B...... | 400  KCl | Oats | 2.10 | 0.97 | 0.09 | 10.0 | 0.06 |
| 2C...... | 4000 CaCO₃ | Oats | 3.70 | 0.49 | 0.20 | 2.5 | 0.87 |
| 3B...... | 400  KCl | Oats | 3.12 | 0.64 | 0.20 | 3.2 | 0.20 |
| 3C...... | 4000 CaCO₃ | Oats | 2.50 | 0.32 | 0.40 | 0.9 | 0.56 |
| 4B...... | 400  KCl | Oats | 3.70 | 0.78 | 0.14 | 5.3 | 0.49 |
| 4C...... | 4000 CaCO₃ | Oats | 3.24 | 0.65 | 0.19 | 3.6 | 0.36 |

tionable than the original acidity. When quantities of potassium held by colloids are small, heavy liming would be necessary to liberate sufficient potash to be of use to plants. Potassium chloride also has been known to increase the alkalinity of soils, especially those

containing lime, as shown by MORSE (16). Analyses given in this report show the variability of potassium in plant tissues and its tendency to depress calcium content. Responses such as these in colloidal soils may account for many apparently contradictory results of numerous investigators on the effect of lime in liberating potassium. The desirable concentration of colloids varies with the chemical content of the soil and the crop to be raised. These conditions in turn should indicate the fertilizer practices to be employed. We need more information on the reasons for the great variability in concentration of elements in soils and tissues, and the rôle of various elements in metabolism in order to determine the kind and quantity of fertilizers to be applied to the soil.

Conclusions derived from chemical examination of tissues without consideration of soil factors are no more reliable than those derived only from soil analyses, and it seems imperative to have thorough knowledge of both soils and crops before conclusions may be made with any degree of reliability. The difference in behavior of clover and cereals on the same soils similarly treated indicates the necessity for knowing fertilizer needs of various crops. It seems safe to assume that these demands vary widely with type of crop and soil. Lysimeter experiments involving study of soil and plants in order to follow changes in them subsequent to use of fertilizers certainly point the way. Not until all factors are known is it judicious to try to select any one of them as an index to others, as it is possible that no factor or nutrient ratio is at all times and under all circumstances predominantly important.

## Summary

1. Crops on acid muck soils chemically deficient in lime or potash may be injured by these elements in fertilizers. Clover is frequently injured by potassium.

2. Potassium increased stem erectness.

3. Use of calcium carbonate as fertilizer depressed the potassium content and the K/Ca ratio in crops grown on acid mucks.

4. High yields of clover showed a low K/Ca ratio in tissues. Crop yield of cereals was not dependent on the value of the K/Ca ratio.

5. Injuries following lime and potash applications to the soil are marked internally by depression of organic nitrogen and carbohydrate content.

6. Antagonism of ions between lime and potash was not observed, that is, neither eliminated the injurious effects of the other.

7. High nitrate nitrogen is associated with high calcium content of tissues and low crop yield.

8. High crop yield is associated with high organic nitrogen and high total carbohydrate content.

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# SALT REQUIREMENTS OF WHEAT AT DIFFERENT GROWTH PHASES

## W. F. GERICKE

### (WITH TEN FIGURES)

Whether or not the salt elements needed for normal growth of vegetation must be present in the culture media of plants for all phases of their development, obviously is of great import in any study that pertains to the relation of the growth of the plant to the composition of the culture media. That the rates of absorption of material by plants change with their progressive development has been shown by numerous experiments. Thus it was found that absorption of inorganic elements by cereals is much greater during the early periods of their growth, when vigorous leaf production prevails, than when the heads form, or even for much earlier growth phases. These circumstances suggest that the composition of the culture media may be markedly altered during progressive growth of plants, without detriment to their ultimate development. To what extent this alteration in the composition of a complete nutrient solution could be extended to comprise a medium devoid of some of the essential elements, obviously would have singular importance in any study of the salt requirement and fertilizer needs of a plant. It occurred to the writer that experiments could easily be conducted that would throw light on this important matter. Plants could be started in complete nutrient solutions, and allowed to grow for a certain time. After having attained certain stages of development, they could be transferred to nutrient solutions devoid of one of each of the elements found in complete nutrient solutions, and there allowed to complete their growth. Likewise, plants could be started in partially complete nutrient solutions, allowed to grow for certain lengths of time, and then could be transferred into the complete nutrient solutions. From such experimentation, cultures would be obtained that were exposed to nutrient media in which one or more of each of the essential elements were absent for different lengths of time and for different stages of development of the plant.
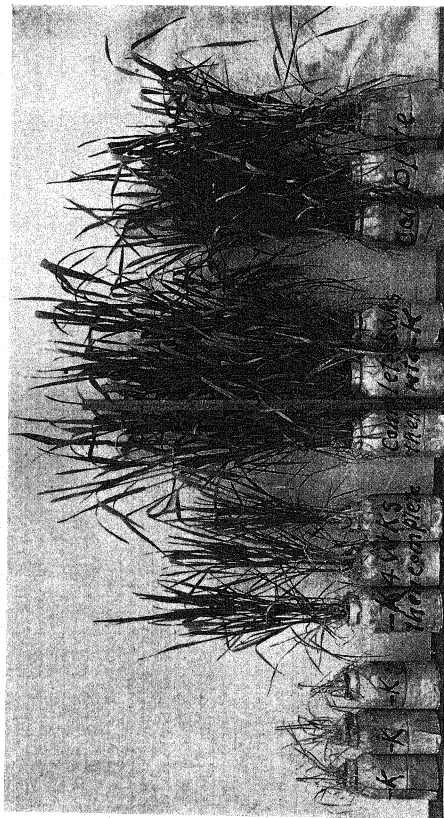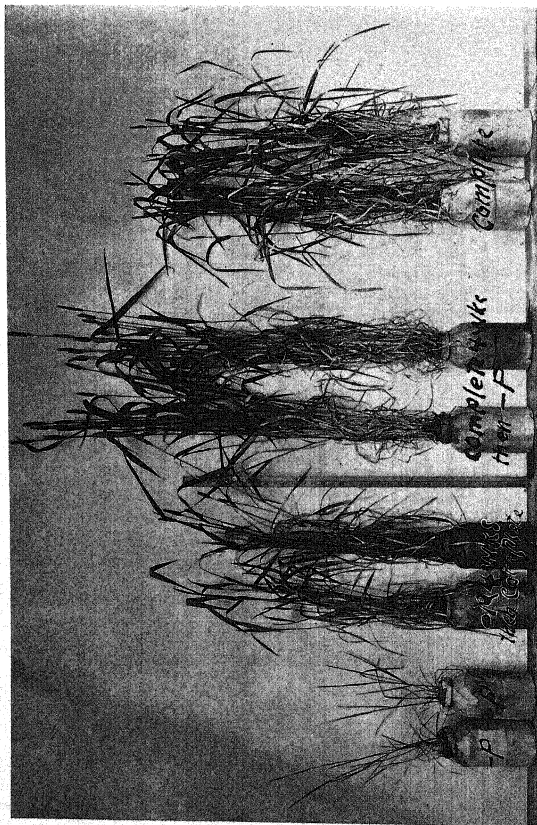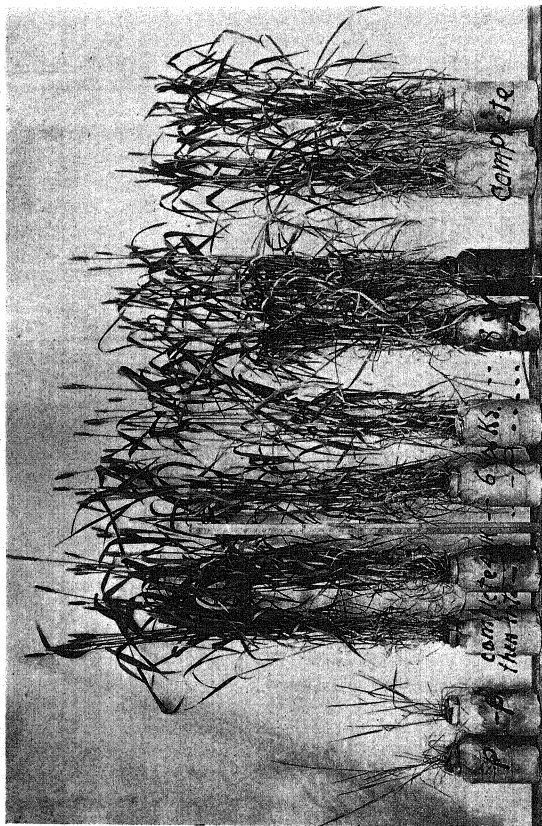
FIG. 1.—Cultures nine weeks old, reading from left to right (in sets of three): set 1, complete, except potassium; set 2, plants grown first four weeks in medium of set 1, and then transferred to complete (set 4); set 3, plants grown first six weeks in complete nutrient solution (set 4), and then transferred to complete growth in solution of set 1; set 4, complete nutrient solution.
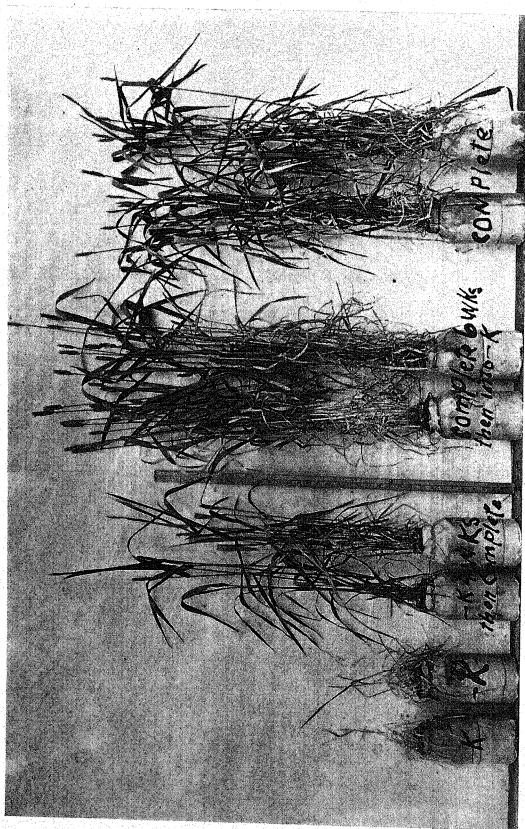
FIG. 2.—Cultures twelve weeks old, reading from left to right (in sets of two); set 1, complete except potassium; set 2, plants grown first four weeks in medium of set 1, and then transferred to complete (set 4); set 3, plants grown first six weeks in complete nutrient solution (set 4), and then transferred to complete growth in solution of set 1; set 4, complete nutrient solution.
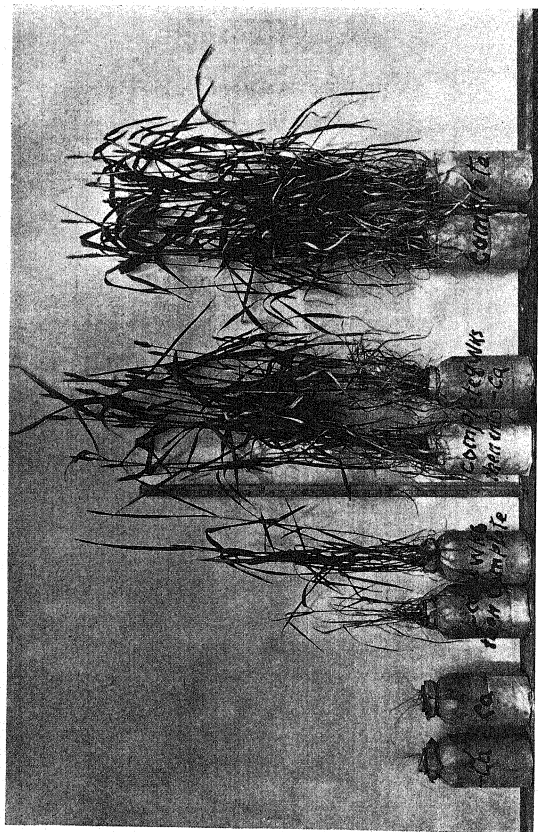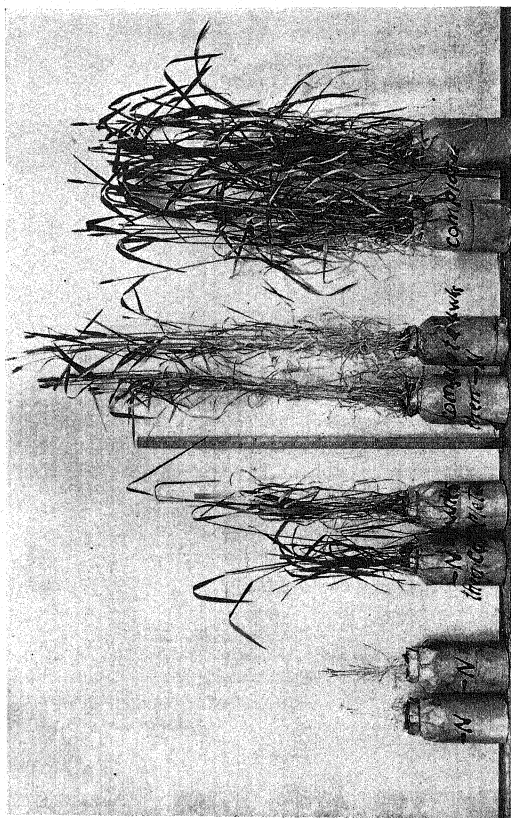
FIG. 3.—Cultures twelve weeks old, reading from left to right (in sets of two); set 1, complete, except calcium; set 2, plants grown first four weeks in set 1, and then transferred to complete (set 4); set 3, plants grown first eight weeks in complete, and then transferred to medium of set 1 (note abnormality of heads), no grain produced; set 4, complete nutrient solution.

This paper contains some of the results obtained from such investigations.[1] Owing to the large number of chemical analyses yet to be made, which are necessary to give full interpretation to the results obtained, this paper will be confined to a presentation of photographs and some data as to weights of plants, to indicate the effects of the treatments on growth.

Briefly, the physiological part of the experiments was as follows: A large number of two-quart Mason jars were filled with culture solutions prepared from the following salts:

(1) Complete nutrient solutions: $KH_2PO_4$, Ca $(NO_3)_2$, $MgSO_4$.
(2) Nutrient solutions minus potassium: Ca $(NO_3)_2$, $MgHPO_4$, $MgSO_4$.
(3) Nutrient solutions minus calcium: $KH_2PO_4$, $KNO_3$, $MgSO_4$.
(4) Nutrient solutions minus iron: $KH_2PO_4$, Ca$(No_3)_2$, $MgSO_4$.
(5) Nutrient solutions minus magnesium: $KH_2PO_4$, Ca$(NO_3)_2$, $CaSO_4$.
(6) Nutrient solutions minus phosphorus: $KNO_3$, Ca$(NO_3)_2$, and $MgSO_4$.
(7) Nutrient solutions minus nitrogen: $KH_2PO_4$, $CaSO_4$, $MgSO_4$.
(8) Nutrient solutions minus sulphur: $KH_2PO_4$, Ca$(NO_3)_2$, $MgHPO_4$.

All nutrient solutions were composed of equal partial molecular concentrations of the salts indicated, and were of osmotic value equal approximately to one atmosphere pressure. The H-ion concentration of these different nutrient solutions varied between $P_H$ 5.7 and 6.7. Wherever adjustments had to be made, salts were used that did not introduce any ions other than those indicated in each of the respective combinations named.

The containers were set with wheat seedlings 4–6 cm. high, and each culture contained five plants. At regular intervals, or when needed, distilled water was added to the cultures to make good the losses that had occurred through transpiration. Likewise iron in small quotas (applications of 0.5 cc. of 0.01 mol. $FeSO_4$) was added at sufficiently frequent intervals to maintain a proper supply of this material to all cultures except those where the plan called for its exclusion.

At different intervals, beginning with four weeks after the seedlings were set, cultures grown in the complete nutrient solutions were

[1] Two papers have appeared: The beneficial effect to wheat growth due to depletion of available phosphorus in the culture media (Science 60:297–298. 1924), and The beneficial effect to plant growth of the temporary depletion of some of the essential elements in the soil (Science 59:321–324. 1924).
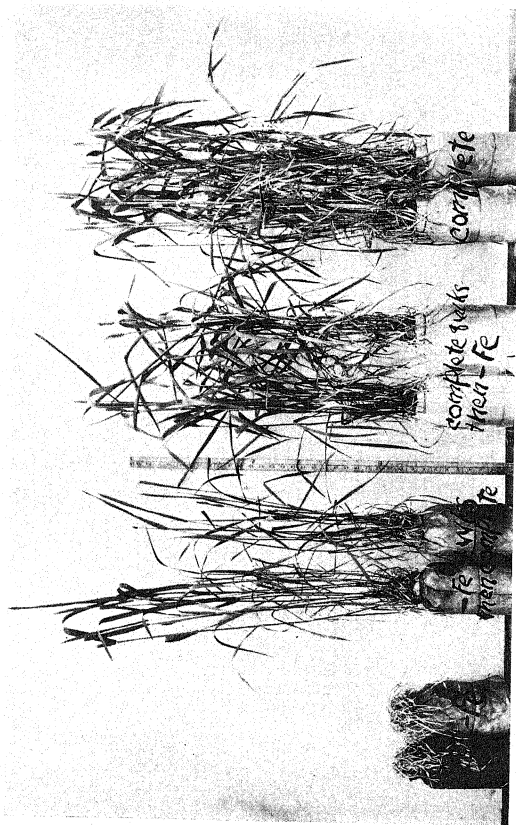
Fig. 4.—Cultures twelve weeks old, reading from left to right (in sets of two): set 1, complete, except iron; set 2, plants grown first four weeks in set 1, and then transferred to complete (set 4); set 3, plants grown first eight weeks in complete, and then transferred to medium of set 1; set 4, complete nutrient solution.
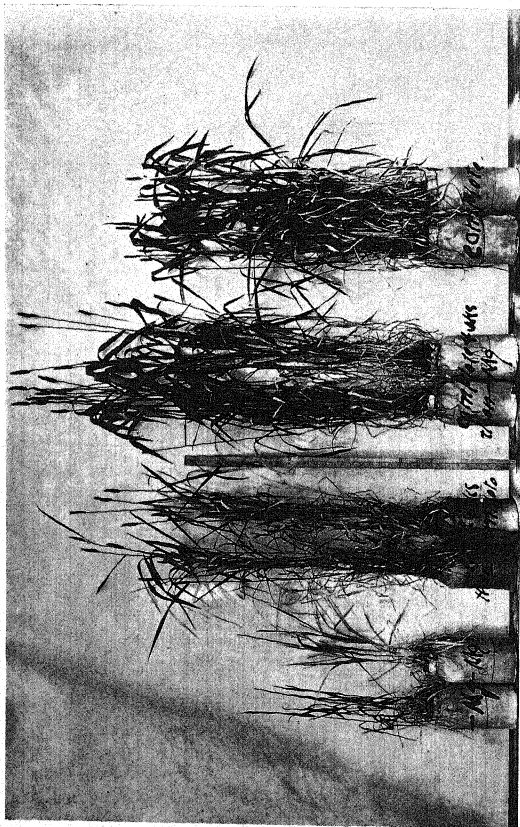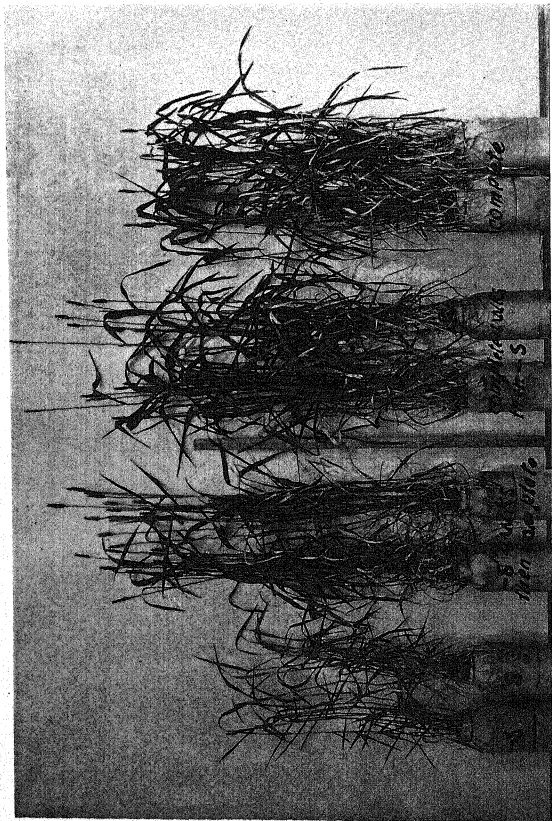
FIG. 5.—Cultures twelve weeks old, reading from left to right (in sets of two): set 1, complete, except magnesium; set 2, plants grown first four weeks in set 1, and then transferred to complete (set 4); set 3, plants grown first four weeks in complete, and then transferred to set 1; set 4, complete nutrient solution.
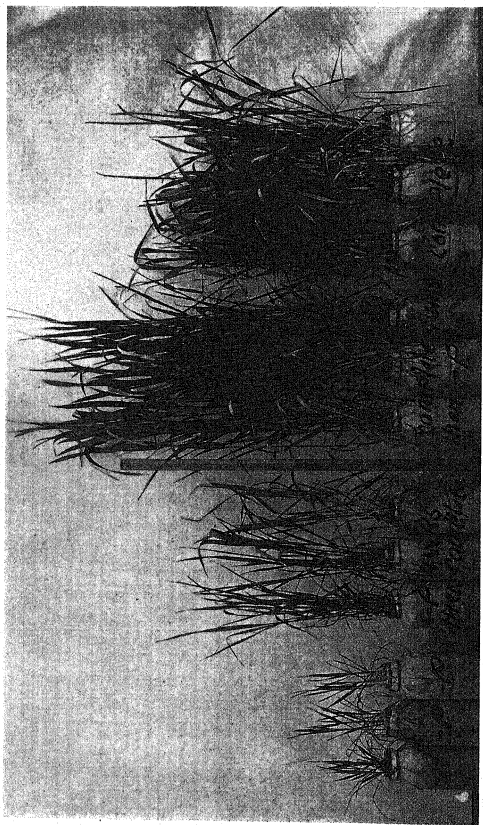
FIG. 6.—Cultures nine weeks old, reading from left to right (in sets of three): set 1, complete, except phosphorus; set 2, plants grown first four weeks in medium of set 1, and then transferred to complete (set 4); set 3, plants grown first four weeks in complete, and then transferred to medium of set 1; set 4, complete nutrient solution.

transferred to the partially complete solutions, and those of the latter to the former. Thus plants grown one month in complete nutrient solutions were placed in culture media in which one of the following elements was absent: K, Ca, Fe, Mg, P, N, and S, and allowed to complete their growth. Likewise, cultures deprived of one of each of these elements for the various fractions of their first growth period, were transferred to media containing the lacking element, and then allowed to complete their growth cycle. In addition to these treatments, two sets of cultures grown in complete nutrient solutions were treated as follows: one was transferred three times into new complete nutrient solutions, and the other was continued in the solution unchanged, in order to have data for comparison of the effect of frequently renewed culture media, that is, media relatively little unchanged by plants, with that changed by plants, because it was not renewed.

The investigation was carried out in the greenhouse. Tests were made of the solutions at intervals to ascertain the reaction of the media, as well as the approximate supply of all elements, in order to preclude any deficiency of elements contrary to the plan as stated. It was found that, in addition to iron, which was rapidly depleted, nitrogen would have been depleted in about 8–10 weeks' growth in the cultures grown in complete nutrient solution unchanged. Consequently, addition of nitrate was made to such cultures before the original supply was exhausted. Because of the very small amount of growth made by the cultures started in the partially complete nutrient solutions, except those devoid of sulphur and magnesium, which made considerable growth (but much less than that of the completes), it is obvious that by the transfer of the cultures from the completes to the partially complete culture media, practically a new supply of material was made available. Hence, with the exception of the cultures started in media devoid of sulphur and magnesium, no additions of nitrate were required to any transferred culture.

The plants were harvested when mature, and the weight of straw, grain, and roots determined. The results are given in table I.

The following conclusions are drawn from the data:

(1) After plants were exposed the first four weeks to complete nutrient solution, and had attained approximately one-seventh of

## TABLE I

DRY WEIGHT OF WHEAT GROWN FIRST IN COMPLETE NUTRIENT SOLUTION, THEN
TRANSFERRED TO NUTRIENT SOLUTIONS CONTAINING ALL ELEMENTS EXCEPT ONE
AS INDICATED (VALUES AVERAGE OF 5 CULTURES, 25 PLANTS PER TREATMENT)

| ELEMENT ELIMINATED FOR EACH SERIES | STRAW (GM.) | GRAIN (GM.) | ROOTS (GM.) | TOTAL (GM.) |
|---|---|---|---|---|
| Set I: grown 4 weeks in complete nutrient solution before being transferred; approximate dry weight when transferred 4.6 gm. | | | | |
| K | 23.9 | 8.4 | 1.3 | 33.6 |
| Ca | 14.8 | 1.6 | 1.3 | 17.7 |
| Fe | 7.9 | ........... | 1.1 | 9.0 |
| Mg | 32.0 | 8.0 | 3.2 | 43.2 |
| PO₄ | 35.4 | 14.0 | 1.8 | 51.2 |
| N | 17.9 | 3.1 | 1.6 | 22.6 |
| SO₄ | 27.4 | 10.0 | 2.3 | 39.7 |
| Set II: grown 6 weeks in complete nutrient solution before being transferred; approximate dry weight when transferred 5.7 gm. | | | | |
| K | 31.2 | 9.5 | 1.2 | 41.9 |
| Ca | 22.1 | .......... | 1.3 | 23.4 |
| Fe | 11.5 | ........... | 1.4 | 12.9 |
| Mg | 29.5 | 8.5 | 3.1 | 41.1 |
| PO₄ | 30.7 | 9.8 | 1.2 | 41.7 |
| N | 18.6 | 4.2 | 1.3 | 24.1 |
| SO₄ | 31.1 | 9.1 | 1.6 | 41.8 |
| Set III: grown 8 weeks in complete nutrient solution before being transferred; approximate dry weight when transferred 8.1 gm. | | | | |
| K | 26.3 | 8.8 | 1.3 | 36.4 |
| Ca | 20.0 | .......... | 2.1 | 22.1 |
| Fe | 18.9 | 1.3 | 1.6 | 21.8 |
| Mg | 32.1 | 9.5 | 2.8 | 44.4 |
| PO₄ | 29.3 | 10.1 | 1.4 | 40.7 |
| N | 19.5 | 3.8 | 2.4 | 25.7 |
| SO₄ | 26.2 | 8.3 | 1.8 | 36.3 |
| Set IV: grown 10 weeks in complete nutrient solution before being transferred; approximate dry weight when transferred 9.5 gm. | | | | |
| K | 24.3 | 6.2 | 1.4 | 31.9 |
| Ca | 18.1 | 4.5 | 1.5 | 24.1 |
| Fe | 25.6 | 2.6 | 1.9 | 30.1 |
| Mg | 30.0 | 5.0 | 2.4 | 37.4 |
| PO₄ | 24.6 | 5.6 | 1.2 | 31.4 |
| N | 19.3 | 4.1 | 2.1 | 25.5 |
| SO₄ | 27.1 | 6.4 | 1.5 | 35.0 |
| Control: average of 10 cultures grown to maturity in complete nutrient solution thrice renewed | | | | |
| | 24.4 | 5.5 | 2.1 | 32.0 |
| Control: average of 10 cultures grown to maturity in complete nutrient solution, not renewed except by addition of 10 cc. of mol. sol. Ca(NO₃)₂ per culture when plants were about 8 weeks old | | | | |
| | 27.9 | 9.4 | 1.7 | 39.0 |

FIG. 7.—Cultures twelve weeks old, reading from left to right (in sets of two): set 1 complete, except phosphorus; set 2, plants grown first four weeks in medium of set 1, and then transferred to complete (set 4); set 3, plants grown first four weeks in complete, and then transferred to medium of set 1; set 4, complete nutrient solution.

FIG. 8.—Cultures twelve weeks old, reading from left to right (in sets of two): set 1, complete, except phosphorus; set 2, plants grown first four weeks in complete, and then transferred to medium of set 1; set 3, plants grown first six weeks in complete, and then transferred to medium of set 1; set 4, plants grown eight weeks in complete, and then transferred to medium of set 1; set 5, complete nutrient solution.

the development (expressed in terms of dry weight) obtainable in such media, and were then transferred to media devoid of magnesium, phosphorus, or sulphur, they produced markedly more grain and straw than did the plants that grew to maturity in complete nutrient solution which was thrice renewed.

(2) Cultures grown six weeks in complete nutrient solution, and having attained approximately one-fifth of the dry weight of produce obtainable from such media, upon transfer to nutrient solutions devoid of potassium, markedly outgrew the cultures kept in complete nutrient solutions which were occasionally renewed. The removal of cultures grown four weeks in complete nutrient solution to a potassium-free medium resulted in dry matter production equal to that obtained from cultures grown in complete nutrient solution which was thrice renewed.

(3) The elements required longest in available form in the growth media for normal development of wheat are calcium and iron. Premature death occurred to the cultures deprived of iron after four to six weeks' growth in complete nutrient solution. Deprivation of iron in cultures grown eight or ten weeks in complete nutrient solution markedly inhibited grain production, the effect being more pronounced in the eight week case than in the ten week case. The removal of cultures (grown four, six, or eight weeks in complete nutrient solution) to media devoid of calcium markedly decreased total dry weight, and practically precluded grain production. The heads produced by the plants deprived of calcium, grown eight weeks in complete nutrient solutions, showed marked abnormalities. Cultures deprived of calcium, grown ten weeks in complete nutrient solution, produced nearly a normal amount of grain, but from its appearance it was judged to be very low in protein.

(4) The removal of cultures grown four, six, eight, or ten weeks in complete nutrient solutions to media devoid of nitrogen curtailed dry matter production, both of grain and straw, but otherwise the plants appeared normal. Good grain, although low in protein, judging from its appearance, was obtained from the cultures which were four weeks old when deprived of nitrogen.

(5) The maximum development among all cultures was obtained from the plants grown four weeks in complete nutrient solution and

FIG. 9.—Cultures twelve weeks old, reading from left to right (in sets of two): set 1, complete, except nitrogen; set 2, plants grown first four weeks in medium of set 1, and then transferred to complete (set 4); set 3, plants grown first four weeks in complete, and then transferred to medium of set 1; set 4, complete nutrient solution.

FIG. 10.—Cultures twelve weeks old, reading from left to right (in sets of two): set 1, complete, except sulphur; set 2, plants grown first four weeks in medium of set 1, and then transferred to complete (set 4); set 3, plants grown four weeks in complete (set 4); set 4, complete nutrient solution.

then transferred into media devoid of phosphorus, in which they grew 104 days.

(6) Better growth was obtained from the cultures grown to maturity in complete nutrient solution which was not renewed, than was obtained from cultures grown in complete nutrient solution which was thrice renewed.

(7) Supraoptimal exposure of cultures to complete nutrient solution of plants transferred to media devoid of K, Mg, P, or S, was inhibitory to maximum development of the plants. The effect of the excess was most noticeable with phosphorus and least with sulphur.

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# DEVELOPMENT OF THE SEED OF ASPARAGUS OFFICINALIS[1]

### W. W. ROBBINS AND H. A. BORTHWICK

#### (WITH FORTY-THREE FIGURES)

In a study[2] having to do with methods of hastening the germination of common asparagus seeds, it became necessary to determine the stages in the development of certain seed structures. This investigation was later extended, and the results are here presented.

The seed of common asparagus arises from an anatropous ovule, two ovules normally being borne in each of the three locules of the ovary. The seed coat is black, finely rugose, and somewhat brittle. The embryo is a slender, threadlike structure completely imbedded in the hard, horny endosperm, the walls of which are hemicellulose. The structure of the mature seed of asparagus is briefly described and figured by HARZ.[3] His descriptions and figures, however, show certain errors, which undoubtedly were due to his failure to follow through the changes which the seed structures undergo in their development. For example, he regards the structureless membrane just beneath the seed coat as the "epidermis of nucellus," whereas developmental studies show this to be of integumentary origin.

Both paraffin sections and sections of fresh seeds were employed. The latter were found to be particularly helpful. All drawings were made with a camera lucida.

## Integument

At the time of pollination, the inner integument of the ovule consists of two cell layers, and the outer integument of from five to ten layers (figs. 1–3).[4] The line of demarcation between the integuments

[1] Contribution from the Division of Botany, College of Agriculture, University of California.

[2] BORTHWICK, H. A., Factors influencing the rate of germination of the seed of *Asparagus officinalis*. Calif. Agric. Exp. Sta., Tech. Paper 18. 1925.

[3] HARZ, C. D., Landwirtschaftliche Samenkunde. 1885.

[4] In all the figures of this paper the following indicators are used: *de*, disintegrating endosperm cells; *e*, endosperm; *em*, embryo; *gr*, stem growing point; *h*, hilum; *ii*, inner integument; *m*, micropyle; *n*, nucellus; *oi*, outer integument; *pe*, pitted, thick walled endosperm; *pm*, pectic membrane; *r*, root tip; *rc*, root cap; *s*, seed coat; *sac*, embryo sac; *sm*, suberized membrane; *su*, suspensor; *te*, thin walled endosperm cells.
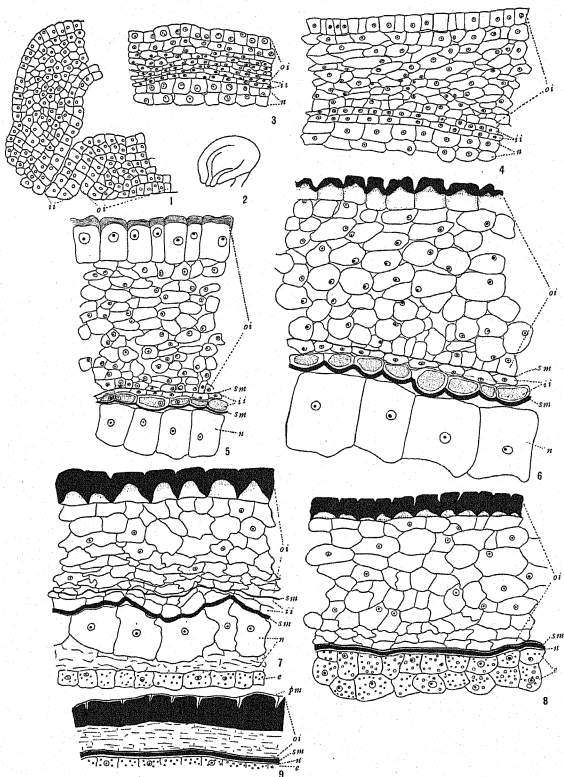
Figs. 1–9.—Sections showing stages in development of seed coat: fig. 1, integuments of ovule at time of pollination; fig. 2, diagram of anatropous ovule; fig. 3, 6 days after pollination; fig. 4, 8 days after pollination; fig. 5, 10 days after pollination; fig. 6, 16 days after pollination; fig. 7, 20 days after pollination; fig. 8, 29 days after pollination; fig. 9, mature seed; thickness of epidermis varies from one part of seed to another; it was not possible in every instance to secure comparable sections from seeds of different ages, a fact which explains in part the discrepancy in size of epidermal cells from seeds of approximately same age; all figs. except no. 2, ×168.

is sharpest near the micropyle. Here inner integument cells are much
larger than they are posteriorly.

Within about sixteen days after pollination, the seed coat has
reached its maximum thickness. The changes during this interval
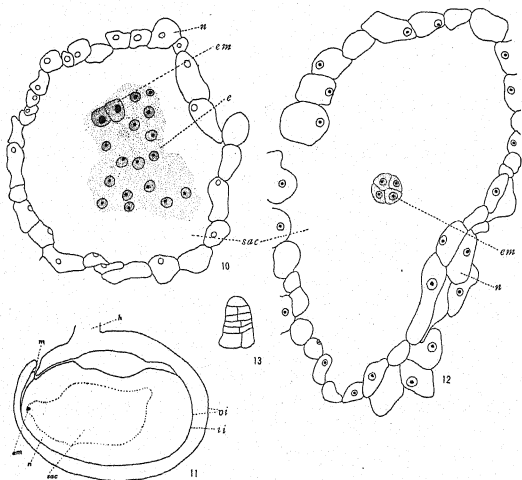


FIGS. 10–13.—Fig. 10, section through micropylar end of embryo sac, 5 days after
pollination; fig. 11, section of fresh seed taken 8 days after pollination; fig. 12, section
through micropylar end of embryo sac 8 days after pollination; fig. 13, embryo dis-
sected from fresh seed; figs. 10, 12, and 13, ×200; fig. 11, ×18.

are shown in figs. 1–6. There is a marked increase in the size of all
cells of both integuments; the outer wall of the epidermal cells shows
pronounced thickening; the two cell layers of the inner integument
become easily discernible from the outer integument; the innermost
cell layer of the inner integument becomes conspicuous because of
the yellowish granular character of its cell contents, and because of
the development of a membrane on its inner wall, adjacent to the
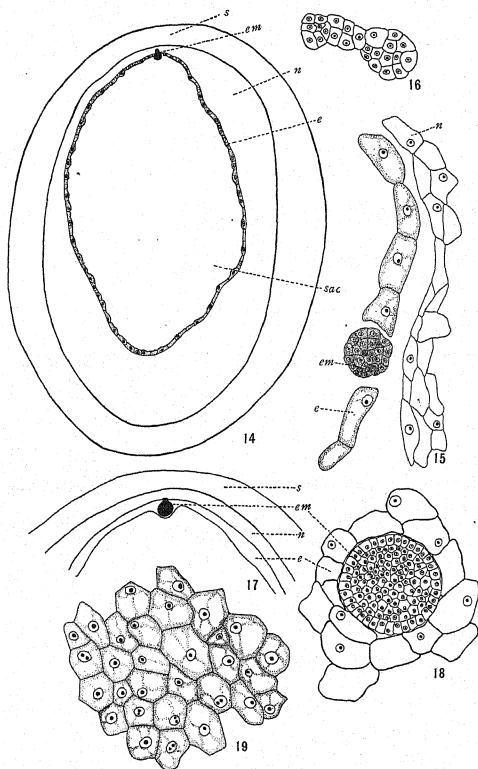
FIGS. 14–19.—Fig. 14, section of seed 13 days after pollination; fig. 15, section through embryo 13 days after pollination; fig. 16, embryo 13 days after pollination; fig. 17, section of seed 15 days after pollination; fig. 18, section of embryo 15 days after pollination; fig. 19, endosperm cells 15 days after pollination; figs. 14 and 17, ×20; all others, ×200.

nucellus; the inner boundary of the outer integument also becomes marked by a thin membrane. These two membranes give a pronounced fat reaction. VAN WISSELINGH[5] has found in the seeds of plants of many families that the two integuments and the innermost integument and nucellus are separated by "cuticles."

In subsequent developmental stages (figs. 7–9) it will be observed that there is a progressive desiccation and shrinkage of the cells, accompanied by compression due to the enlarging endosperm, with a consequent decrease in the thickness of the seed coat. The outer wall of the epidermal cells becomes very thick, the lumina of these cells being almost completely filled with a dark brown substance. Covering the outer surface of the epidermal cells is a thin, transparent membrane (fig. 9), which stains red with ruthenium red and violet with methylene blue. These color reactions indicate a pectic substance. This hydrophilous membrane which covers the surface of the seed probably facilitates the absorption of water.

By about the thirtieth day following pollination the two fatty membranes are closely compressed (fig. 8). This condition has been brought about by the disintegration of the cells of the inner integument, accompanied by pressure on both sides. In the mature seed the membranes are usually indistinguishable, unless the tissues are treated with a solution of potassium or sodium hydroxide to bring about their swelling and separation.

This double membrane gives the following microchemical reactions: with Sudan III, a deep orange-red color; with methylene blue, a dark blue color; and with basic fuchsin, a red color, none of these colors fading in dilute acids or alcohol; ready dissolution when boiled in 2 per cent potassium hydroxide solution; and insolubility in 50 per cent chromic acid. That the membrane is of a fatty nature is well borne out by these tests. Studies, to be reported elsewhere, show that the semipermeability of the seed coat of asparagus is localized in this membrane.

### Nucellus

Contemporaneous with the enlargement of the seed coat, there is an increase in the size of nucellar cells. These attain their maxi-

[5] VAN WISSELINGH, C., Bijdragen tot de Kennis van de Zaadhind. Pharm. Weekblad 56:849–868; 1246–1271. 1919.
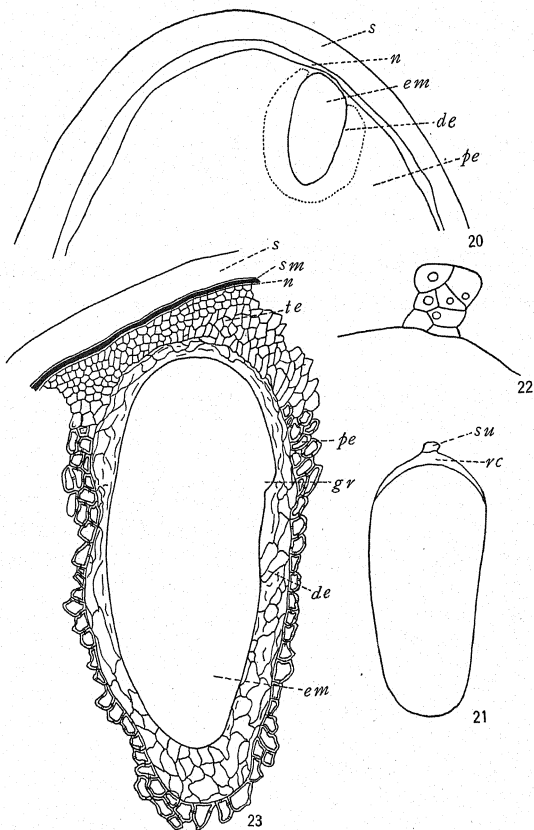
FIGS. 20–23.—Fig. 20, section of micropylar end 20 days after pollination; fig. 21, embryo 20 days after pollination; fig. 22, suspensor 20 days after pollination; fig. 23, section of seed 29 days after pollination, showing thin walled endosperm cells (*te*) in front of root tip, compressed and disintegrating endosperm cells (*de*) surrounding enlarged embryo, and pitted, thick walled endosperm cells (*pe*); fig. 20, ×31; figs. 21 and 23, ×62; fig. 22, ×330.

mum size about the sixteenth day after pollination. From this time
the nucellar tissue becomes gradually disorganized and absorbed,
its disappearance being initiated in the region bordering the endo-
sperm (fig. 7). By the twentieth or twenty-first day, the encroach-
ing endosperm has reduced the nucellus to a very narrow band of
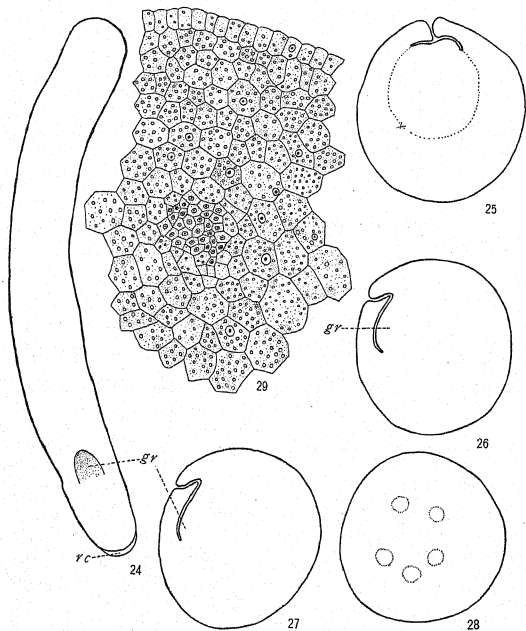


FIGS. 24–29.—Fig. 24, external view of mature embryo; figs. 25–27, cross-sections
through stem growing point; fig. 28, cross-section through cotyledon, showing 5 pro-
cambium strands; fig. 29, cross-section through portion of cotyledon, showing single
procambium strand, and all cells filled with fat globules and protein granules; fig. 24,
×33; figs. 25–28, ×54; fig. 29, ×275.

compressed cells. Within about one month after pollination the nucellus is represented by a highly attenuated structureless layer, recognizable with difficulty unless properly stained (figs. 8, 23, 30).
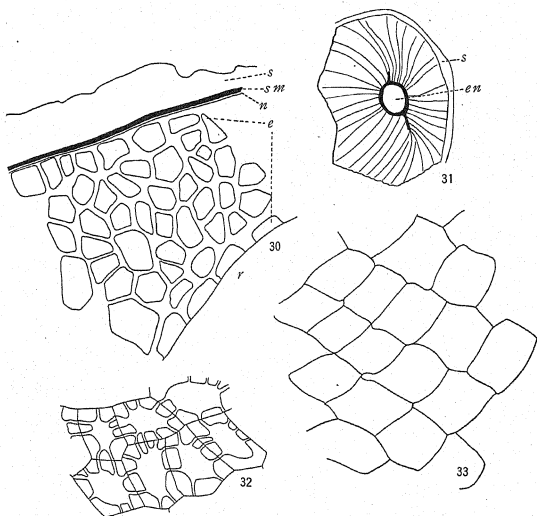


FIGS. 30–33.—Fig. 30, section of mature seed, showing endosperm tissue just in front of root tip; fig. 31, diagram of section of mature seed, showing relation of embryo (*em*) to surrounding compressed and disorganized endosperm cells (solid black), radiating lines indicating radiating rows of endosperm cells; fig. 32, ordinary endosperm cells with thickened hemicellulose walls, middle lamella, and pits; fig. 33, endosperm cells after having been treated with 55 per cent sulphuric acid for 10 minutes; hemicellulose walls dissolved, leaving middle lamellae; ×260.

In the mature seed, the fragmentary remains of the nucellus show characteristic microchemical reactions. When a section of the seed is treated with 50 per cent $H_2SO_4$, the nucellus is the first to dissolve. With methylene blue, it gives a pronounced violet color,

and with ruthenium red a red color. These reactions indicate that the nucellus of the mature seed is pectic in nature.

## Endosperm

The earliest stages in the development of the endosperm were not observed. On the fifth day following pollination, the endosperm was seen to be composed of a relatively small number of free nuclei, at the micropylar end of the seed (fig. 10). Rapid nuclear division ensues, and by the tenth day after pollination, endosperm nuclei are distributed quite uniformly throughout the peripheral region of the embryo sac (fig. 38), imbedded in a thin layer of cytoplasm. It is not until about the thirteenth or fourteenth day, however, that wall formation between endosperm nuclei begins (figs. 14, 15). Endosperm development now proceeds centripetally at a rapid rate, encroachment upon the embryo sac being approximately uniform from all sides. About three weeks after pollination, the endosperm tissue completely fills the embryo sac. At this time the embryo is relatively small (fig. 42). The storage of the endosperm with food reserves begins when the tissue is completely formed, and continues until the seed is ripe. The reserves are hemicellulose in the walls, and oil and protein in the lumina. Increase in the thickness of the walls of endosperm cells is first observed about the twenty-fifth day after pollination. Cell wall thickening takes place first in the innermost cells, and progresses centrifugally. As the walls thicken narrow canals are left at intervals, those of adjoining cells coinciding (fig. 32). Endosperm cells adjacent to the nucellus remain thin walled until the seed is almost mature.

Microchemical studies show that the middle lamellae of endosperm cells are of pectic substance, whereas the thickened walls are of hemicellulose. Forty-five per cent sulphuric acid dissolves the hemicellulose walls somewhat rapidly, 3 per cent sulphuric acid at room temperature slowly dissolves them, and 3 per cent sulphuric acid at boiling temperature dissolves the walls completely in from five to ten minutes. Chromic acid also causes slow dissolution. With methylene blue the thickened walls are stained a light blue, and stand out in marked contrast with the dark violet-blue of the middle lamellae. The middle lamella is stained red with ruthenium red, while the thickened portion of the wall is unstained.

The endosperm throughout is not homogeneous in its structure and chemical constitution. In sections cut parallel with the embryo, a fan-shaped group of endosperm cells spreads out from the root tip to the seed coat. These cells differ from the great mass of endosperm cells, and are characterized by their irregular cubical shape, the absence of pits, and the fact that the walls stain a very deep violet with methylene blue and pink with ruthenium red, indicating pectic material. The presence of this tissue, rich in pectic material, between the root tip and the seed coat, appears to be of advantage to the growing embryo at the time of germination. Such tissue not only absorbs water readily, but it is one through which the root tip can find comparatively easy penetration. In any section of the seed (taken about one month after pollination) which includes the embryo, the tissue immediately surrounding it is composed of endosperm cells in various stages of disorganization (fig. 23); it becomes structureless and apparently composed only of cellular membranes. There are also groups of cells radiating from the embryo (fig. 31) which are very thin walled, compressed, and partly disorganized. The walls of these cells, and also of the structureless tissue, give
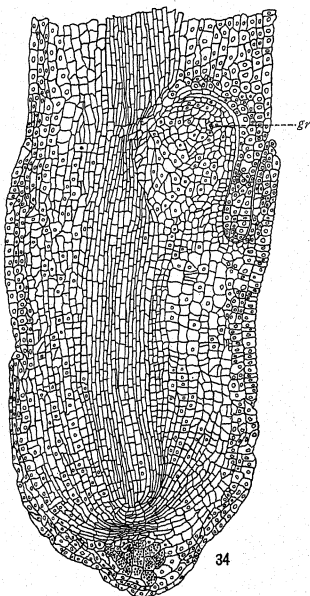


Fig. 34.—Longitudinal section of tip of embryo from mature seed; ×100.

color and solubility reactions which indicate that they are pectic substances.

## Embryo

The earliest stage observed in the development of the embryo was as shown in fig. 10. About ten days after pollination, the proembryo could be observed in suitable sections of the fresh berry. Its position in the seed, and its size, relative to other seed structures, are seen in fig. 11. At this age the proembryo consists of an irregular shaped, rather massive suspensor of the *Lilium* type, and a few celled embryo. The suspensor is partly surrounded by nucellar tissue, and its attachment to the nucellus is firm enough to prevent the proembryo from being easily shaken loose in the ordinary handling of fresh sections on the microscope slide. Between the tenth and thirteenth day, the suspensor elongates somewhat, pushing the rapidly enlarging embryo into the cavity of the embryo sac (figs. 14, 17). By the second week the developing endosperm has completely surrounded the free end of the embryo (fig. 17). At no subsequent stage of its development does the embryo project freely into the cavity of the embryo sac. By the third week (figs. 20–23) the embryo has become an elongated, many celled structure. The root cap is visible, and a number of its cells possess starch grains. The suspensor is still attached to the embryo. As stated, immediately surrounding the enlarging embryo are rows of thin walled, compressed endosperm cells which by their appearance and behavior are different from the endosperm cells farther removed from the embryo. When fresh sections of the seed are first placed in water and examined, these cells are not noticeably different from other endosperm cells, but after standing in water for half an hour, there is a disorganization of the cells, the walls apparently dissolving, and all that remains are small aggregations of proteinaceous and fatty material.

During the fourth week there is a rapid increase in the size of the embryo. It becomes freed from the nucellus and completely invested by the endosperm. A lateral depression develops a short distance posterior to the root cap, within which the stem growing point is situated (fig. 23). The hypocotyl is short, and the cotyledon elongates so that the mature embryo is almost the full length of the seed. During germination the cotyledon remains in the seed in close
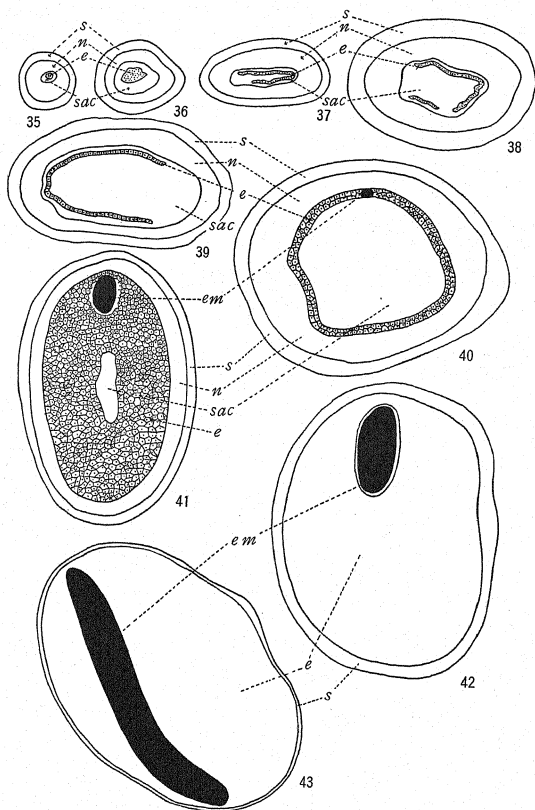
FIGS. 35–43.—Diagrams of 9 stages in development of seed: fig. 35, 5 days after pollination; fig. 36, 6 days after pollination; fig. 37, 8 days after pollination; fig. 38, 10 days after pollination; fig. 39, 13 days after pollination; fig. 40, 16 days after pollination; fig. 41, 20 days after pollination; fig. 42, 29 days after pollination; fig. 43, 44 days after pollination; ×15.

contact with the endosperm, acting as an absorbing and conducting organ.

It will be observed from an examination of figs. 23, 41, and 42 that the developing embryo is confronted with a thick walled endosperm tissue, through which it must make a path. It has already been pointed out that the endosperm cells immediately in front of the embryo appear to be in a state of disorganization; the walls of such cells give reactions indicating pectic substance. This situation continues throughout the growth of the embryo.

### Mature seed

In the mature seed, the seed coat has a single, epidermal layer of cells, the outer wall of which is thick, the inner thin, and the lumina are almost completely filled with a dark brown material. A thin, transparent pectic membrane covers the outer surface of the epidermal cells. Beneath the epidermis are rows of highly compressed cells, the lumina of which appear as mere dark slits. Below these cells is a suberized membrane, separable into a thin and a thick membrane, closely compressed. These represent, respectively, the "cuticles" of the inner and outer integuments. Under the fatty double membrane is a narrow structureless zone, giving a pronounced pectic reaction, which represents the remnants of nucellar tissue. The bulk of the seed is composed of endosperm cells which have thick pitted walls of hemicellulose, and lumina well filled with protein granules and fat globules. The endosperm cells immediately surrounding the embryo, and also narrow strands of cells radiating from the embryo, are much compressed, and in various stages of disorganization. Endosperm cells between the root tip of the embryo and the nucellar zone are relatively thin walled and pitless, and the walls appear to be largely pectic in nature.

The mature embryo is a slender, threadlike structure completely imbedded in the endosperm. It is differentiated into root, with root cap, short hypocotyl, stem growing point, and elongated cotyledon.

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# CONTINUITY OF PROTOPLASM IN ENDOSPERM CELLS OF DIOSPYROS[1]

EDUARDO QUISUMBING

(WITH PLATES XXIII, XXIV AND TWO FIGURES)

## Introduction

This paper is an account of the results of a study of protoplasmic connections in the endosperm cells of *Diospyros discolor, D. ebenaster, D. Ahernii,* and *D. kaki.* The investigation was begun at the University of Chicago, and was completed in the Philippines.

Methods were developed for demonstrating protoplasmic connections in the endosperm cells of *D. discolor, D. Ahernii, D. ebenaster,* and *D. kaki.* Various attempts to perfect an indirect method for demonstrating continuity of protoplasm in vegetative and reproductive tissues have been only partially successful, as was found by GARDINER (3), TANGL (15), and many others, and also by the writer. While the present method proved to be satisfactory for the endosperm of *Phytelephas macrocarpa, D. discolor, D. Ahernii, D. ebenaster,* and *D. kaki,* it is unsuitable for the endosperm of some seeds, as, for example, *Cocos nucifera.* This difference in reaction is due to the chemical nature of the endosperm cells, and also to the thickness of the cell walls. The discovery of protoplasmic connections in the endosperm of all seeds would be important both for the physiologist and morphologist, as the results might throw some light on some of the complicated problems of the physiology of seeds.

Protoplasmic connections are known to occur in vegetative as well as in reproductive tissues, and have been observed in all the great divisions of plants, even in the lower Thallophytes. They have been described in the Cyanophyceae, fungi, and lichens. They have also been observed in *Volvox, Gonium, Spirogyra, Cladophora, Ulothrix, Mesocarpus,* and the Phaeophyceae. Continuity of protoplasm has been demonstrated in the Rhodophyceae, especially in the Florideae, in liverworts, in moss leaves, guard cells and stem, in Pterido-

[1] Read before the Los Baños Biological Club, October 28, 1924.

phytes, and in gymnosperms. In angiosperms the occurrence was proved by GARDINER (1–7), KUHLA (11), and many others. They occur in parenchyma cells of vegetative tissues, xylem and phloem, in nodes of grasses, and motile organs of the leaves, etc.

Although a voluminous literature exists on the continuity of protoplasm in vegetative tissues, there is very little on reproductive tissues, particularly the endosperm. TANGL (15) first demonstrated the existence of protoplasmic connections in ripe endosperm cells of *Strychnos nux-vomica*, *Areca oleracea*, and *Phoenix dactylifera*. He found that the cell walls in these species were perforated by fine protoplasmic threads. Six years later he (16) investigated the endosperm of grasses, and found that the aleurone and starch cells are in mutual connection by means of very fine protoplasmic threads passing through unpitted cell walls. MOORE (12) investigated the continuity of protoplasm through the cell walls of endosperm cells in several species of *Strychnos*, and of *Diospyros embryopteris* and *D. melanoxylon*. GARDINER'S (3) work on the protoplasmic connections in seeds appeared in 1883. He examined a number of monocotyls, especially palms, numbering fifty-three species, and dicotyls numbering twenty-nine species represented by eleven families. He pointed out also that the structure of the protoplasmic connections in various seeds presents every possible modification. He (5) studied the technique for demonstrating protoplasmic connections, and in 1897 published his results on *Tamus communis*, and also the interesting structure of the endosperm cells of *Lilium Martagon*. Others who have described protoplasmic connections in the endosperm are GARDINER and HILL (8, 9); STRASBURGER (14) who described and figured protoplasmic connections in the endosperm cells of *Phytelephas macrocarpa* and *Tamus communis;* KENITZ-GERLOFF (10); ZABRISKIE (19); STOPES and FUJII (13); and WULFF (17, 18). Besides these investigators, many have mentioned and figured protoplasmic connections of the endosperm in textbooks.

## Development of technique

The history of the development of technique for demonstrating protoplasmic connections is so voluminous that only the principal methods can be mentioned.

TANGL (15), who first announced the existence of protoplasmic connections in Phanerogams in his work on the endosperm of *Strychnos nux-vomica*, *Areca oleracea*, and *Phoenix dactylifera*, treated his sections with iodine diluted with alcohol or with potassium iodide, and then mounted them in chloriodide of zinc. The connections stained dark brown and the cell walls yellow. He tried to use haematoxylin and carmine after fixing in iodine, but failed. He also treated his material with tincture of iodine. GARDINER (3) saw the threads clearly in the endosperm cells of *Bentinckia Conda-panna* by mounting sections in dilute glycerine. In the case of *Tamus communis*, he used osmic acid-uranium nitrate mixture (Kolossow's reagent) as a fixative, and preserved in thymol water for future use. Safranin alone or Hoffmann's blue was used for staining. Safranin may be followed by gentian violet or eosin. GARDINER also tried sections of endosperm, placed in water, treated with iodine, and mounted in chlorzinc iodide; washed in water stained with picric-Hoffmann's blue; mounted in glycerine or glycerine jelly. For *Strychnos* and *Tamus* he modified this method by placing sections in alcohol and treating with alcoholic iodine and mounting in glycerine. MOORE (12) reported seeing protoplasmic threads in the seeds of *Strychnos Ignatia* without fixing or staining, by simply placing sections of the hard dark endosperm in water. He succeeded in staining the threads better, however, by placing the sections in picric acid, washing, and then leaving them in Judson's Oxford blue for a few minutes, or washing in water or in strong or dilute glycerine. He showed threads in *Diospyros embryopteris* and *D. melanoxylon* by placing sections in chloriodide of zinc for twenty-four hours, and then washing, and staining them in picric blue. STOPES and FUJII detected "plasmodesma" in *Encephalartos Lehmanni*, *Zamia floridana*, and other material by using hand sections of alcoholic material, washing in water, treating for a short time with sulphuric acid, and then staining in aniline blue.

## Procedure

Ripe seeds of *Diospyros kaki* were furnished through the courtesy of Dr. SHIGEO YAMANOUCHI of Tokyo Higher Normal School, Japan. These were preserved in ordinary formaldehyde, and were

washed in 50 per cent alcohol before cutting. The seeds of *D. dis-color*, *D. kaki*, *D. Ahernii*, and *D. ebenaster* were cut fresh and also after preserving in formo-aceto-alcohol, or in 10 per cent formalde-hyde.

The first difficulty encountered is the method of cutting, as all the seeds are more or less bone-hard. It was found also that the planes cut affected the ease of examining protoplasmic connections after mounting. The writer found exceptions in the case of *D. dis-color* and *D. Ahernii*, where all planes showed perfectly clear proto-plasmic connections. Of the four species of *Diospyros* so far studied, *D. discolor* and *D. Ahernii* were similar to *Strychnos Ignatia*. Proto-plasmic connections may be seen in sections mounted in water under a high power dry objective.

<p align="center">TECHNIQUE</p>

A Spencer sliding microtome or the rotary microtome was used for sectioning. Longitudinal radial sections of seeds (text fig. 1) show the best view for studying protoplasmic connections. In transverse sections (text fig. 2) the cells are elongated, except in *D. Ahernii*, in which the cells are isodiametric. *D. discolor* and *D. Ahernii* show the protoplasmic connections best, both in transverse and radial longitudinal sections.

Before the seeds are clamped on the microtome holder, they should be trimmed as illustrated in text figs. 1 and 2. The sections may be cut 5–15 $\mu$, lifted with a camel's hair brush, and transferred into a dish containing the fixative. Fix in Bouin's fluid for forty-eight hours, and then wash in 50 per cent alcohol until the yellow color disappears from the liquid and the sections after washing. Two methods may be given for the next step.

1. From 50 per cent alcohol transfer the sections to alcoholic safranin and allow to stay for a day. Then wash sections in water to remove extra safranin, and stain further in aqueous gentian violet for four days; wash in water, pass through grades of alcohol (50, 95; and 100 per cent) at intervals of two minutes, then through clove oil and xylol, after which they may be mounted in Canada balsam. Safranin is prepared by dissolving 10 gm. of safranin crystals in 500 cc. of 95 per cent alcohol. The container is shaken and allowed

to stand over night. The next day the stain is transferred in a liter flask and filled up to the neck with distilled water, and then filtered. Gentian violet is prepared by dissolving 5 gm. of the crystals in 500 cc. distilled water. After shaking thoroughly, and allowing to stand for a day, it is filtered.

2. The best stain for demonstrating protoplasmic connections in the endosperm cells of *Diospyros* is the Haidenhain's iron-alum haematoxylin. After washing the sections in 50 per cent alcohol, they should be transferred to water, passing through 30 and 10 per cent alcohol with ten minutes in each grade. All trace of alcohol should be washed out before putting sections in iron-alum. The sections should be placed in 2 per cent iron-alum for two hours, washed thoroughly in water, and then placed in 0.5 per cent haematoxylin over night. Haematoxylin used should ripen six weeks from the date it is prepared; very old haematoxylin is unsatisfactory. Sections are washed thoroughly in water the next morning, transferred to iron-alum, and then washed thoroughly in water before dehydrating. After washing use different grades of alcohol (10, 30, 50, 80, 95, 100 per cent) at five to ten minute intervals. The sections are then placed in gold orange dissolved in clove oil for about five minutes, into clove oil, and then into xylol, from which they are mounted in Canada balsam. Sections of endosperm from young seeds collapsed and shriveled when placed in xylol, so they were mounted in glycerine jelly after staining.

FIGS. 1, 2.—Fig. 1, front view of seed, showing method of obtaining longitudinal radial sections; fig. 2, side view, showing method of obtaining transverse sections.

## Seeds

*Diospyros discolor* is endemic to the Philippines. The fruit is edible, and is covered by densely pubescent purplish brown hairs. The seeds are ellipsoid, one side flat and the other convex, 3–3.2 cm.

long, 2–2.3 cm. wide, and 1.3–1.5 cm. thick. The seed coat is thickest at the micropylar end (0.5–0.75 mm.), light reddish brown externally, but differentiated internally into a dark brown layer. The endosperm is white and hard, resembling that of *Phytelephas macrocarpa*, although a little softer.

*Diospyros ebenaster* is an introduced species, coming from Mexico during the early colonial period. It is now being cultivated, and is noted for its large edible fruits, which are smooth and green when mature, but turn brown when ripe. The edible pulp is yellowish, but soon turns black when ripe. The seeds are oblong, compressed, one side keel-shaped and the other thickened and more or less rounded, 2.2–2.5 cm. long, 1.2–1.5 cm. wide, and 7–9 mm. thick. The seed coat is thin and reddish brown in color. The endosperm is cream white, and in all essentials like *D. discolor* in texture.

*Diospyros Ahernii* is an endemic species. The fruits are pubescent, coated with yellow hairs. The pulp is thick and fibrous, and not very palatable. The edible portion of the fruit is the sweet gelatinous mass which covers the seeds. The seeds are shaped like *D. discolor*, with one side flat and the other convex, 2.5–2.6 cm. long, 1.0–1.3 cm. wide, and 8–10 mm. thick, orange brown in color. The seed coat is thin like that of *D. ebenaster*. The endosperm is white and somewhat hard in texture.

*Diospyros kaki* is a Japanese species. A few plants of this species are being cultivated in the Philippines, in the mountain province, island of Luzon. The seeds are hemi-ellipsoid to ellipsoid, 1.8–2.5 cm. long, 0.9–1.2 cm. wide, and 4–6 mm. thick. The seed coat is little thicker than that of *D. ebenaster*, but the color and texture of the endosperm resemble that species.

ENDOSPERM CELLS

The seed is invested by a coat of several layers of parenchymatous cells (fig. 1), forming a more or less spongy testa. Beneath this coat the outermost cells of the endosperm are smaller and thinner than the underlying ones. The nuclei persist in nearly all of the cells.

The form and size of the endosperm cells are different in different species, and in different parts of the same endosperm. As previously

indicated, the cells are almost isodiametric in radial longitudinal sections of the endosperm, and are elongated horizontally in transverse sections, except in *D. Ahernii*, where all cells are isodiametric.

The following description of the endosperm cells is for *D. discolor*. It holds true for the other species studied with little variation, except *D. Ahernii*. The cells in the other species vary in size and thickness of the walls. In transverse sections of the endosperm (fig. 1) the form and size of the cells vary as the axis of the seed is reached. The cells adjacent to the coat are smaller and somewhat isodiametric, although the epidermal cells are more or less squarish. Some, however, are slightly elongated horizontally, and the corners are rounded. The cell walls are thinnest near the coat (6–10 $\mu$), and thicken gradually toward the underlying cells. Thin walled cells may be seen also in the middle. No striations of any kind are evident in the walls. The nucleus is present and persists in the cells, near the periphery, but seems to be disorganized in the cells within. A great number of tiny rounded granules are imbedded in the cytoplasm. In *D. ebenaster* the cell inclusions are these tiny granules, but in addition there are large rounded globules of oil. The middle lamella is very prominent in cells near the periphery, but is present in all the cells. It is perforated by the protoplasmic connections. Three or four layers away from the periphery in transverse sections the cells begin to elongate, and are at their best several layers within, some even attaining the length of 130 $\mu$, with tapering ends. The walls are 8–14 $\mu$ thick. The cells near the axis of the seed are less elongated, more or less isodiametric, with sharp corners and thickened walls, sometimes attaining a diameter of 18 $\mu$.

In radial longitudinal sections the endosperm cells are nearly always isodiametric, except in *D. Ahernii*, with more or less rounded lumen, with the exception of those cells near the periphery, which in form and size resemble those of the transverse sections. The diameter of the lumen varies even in the same species.

### Protoplasmic connections

Protoplasmic connections in the endosperm cells of *Diospyros* species studied may be classified under two main categories, those which occur in great numbers and those which are scanty, isolated,

and restricted. Of the four species studied, protoplasmic connections may be observed in two species without the aid of any fixative or stains, by simply mounting the sections in water. They are extremely delicate in *D. kaki* and in *D. ebenaster*, but are quite pronounced and thickened in *D. Ahernii* and *D. discolor*. Protoplasmic connections at the ends of the groups are generally thicker than those in the middle, measuring 0.5–0.55 $\mu$. Some connections in the middle are also thickened like those at the ends, and this is especially true in the three species except *D. discolor*, which sometimes shows such thickening also at the ends. The thickness of the connections is exaggerated in the photomicrographs (figs. 7–10). The doubling situation of connections appears single in the photomicrographs.

The number of protoplasmic connections traversing the whole thickness of the unpitted cell walls varies according to the species. They are very numerous in *D. discolor* (figs. 1–3, 7), and in *D. Ahernii* (figs. 4, 8). The arrangement of the protoplasmic connections is well demonstrated in the elongated cells of *D. discolor* (figs. 1, 2). In *D. ebenaster* (figs. 5, 9) the protoplasmic connections crossing the walls are not so numerous, the minimum number being six. *D. kaki* has the least number of the four species (figs. 6, 10), sometimes as low as four traversing the walls. In all cases the number and identity of the protoplasmic connections seem to disappear toward the periphery (fig. 1). Very few protoplasmic connections are present in walls of cells near the coat.

The length of protoplasmic connections seems to vary greatly, and this variation is due to the thickness of the walls to be traversed. Protoplasmic connections away from the periphery are usually longer than those found traversing the walls of cells near the periphery, or thin walls anywhere. The form of the protoplasmic connections varies also. Those at the ends of groups are generally rounded or acute. The ones at the center are usually straight, and in elongated cells they are sometimes oblique in position. Protoplasmic connections occur either singly or in groups. When single they are generally thicker than those in groups. Groups of two are very common, while three, four, five, or six are rare, especially the five and six. Sometimes the protoplasmic connections coalesce, and when this occurs the connections seem to be very much thickened. In *D. discolor* and

*D. Ahernii* protoplasmic connections occur over the whole area of the cell walls. In *D. ebenaster* and *D. kaki* they are restricted to the middle of the cell walls, and very seldom occur at the corners. In none of the cases have any nodal swellings of the protoplasmic connections been observed. The groupings of the protoplasmic connections in *D. kaki* and *D. ebenaster*, and sometimes in the two other species, result in a barrel-shaped form.

### Summary

1. Protoplasmic connections in *D. discolor* and *D. Ahernii* may be observed without the aid of fixation and stain.

2. The use of Bouin's fluid for killing and fixing the endosperm of *Diospyros* seeds is very satisfactory.

3. Haidenhain's iron-alum haematoxylin, counter stained with gold orange dissolved in clove oil, proved to be the best combination of stains. The cell walls were colored orange and the protoplasmic connections dark bluish brown.

4. Safranin gentian violet stains also proved satisfactory. The cell walls were colored pinkish red, with the protoplasmic connections violet.

5. The nature of the protoplasmic connections in the endosperm cells of *Diospyros* species studied is not at all similar. In *D. discolor* and *D. Ahernii* they are very numerous, and are found perforating the unpitted cell walls throughout; in *D. kaki* and *D. ebenaster* they are few, restricted, and grouped at the walls. They occur singly, or in groups of two, three, four, five, or six, and are thicker when single and usually thinner in groups.

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QUISUMBING on DIOSPYROS
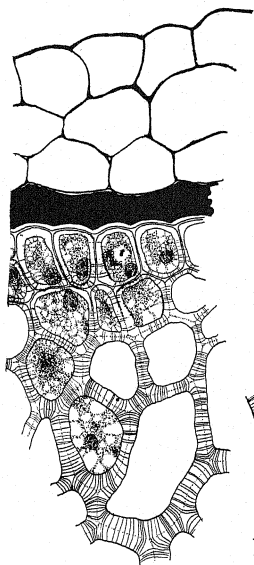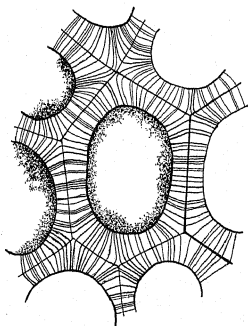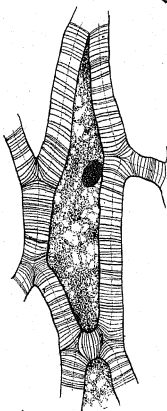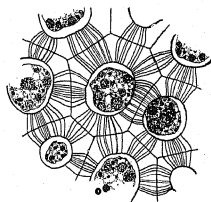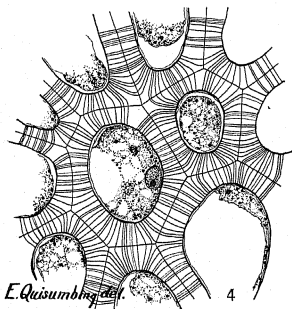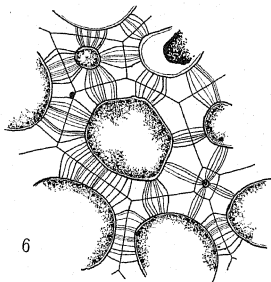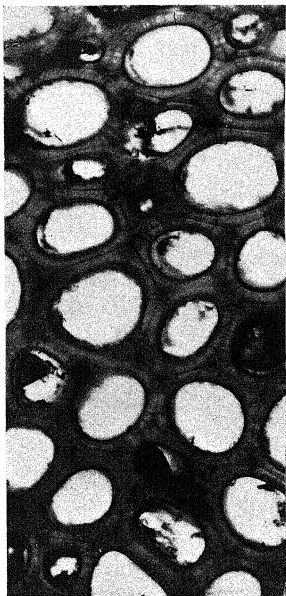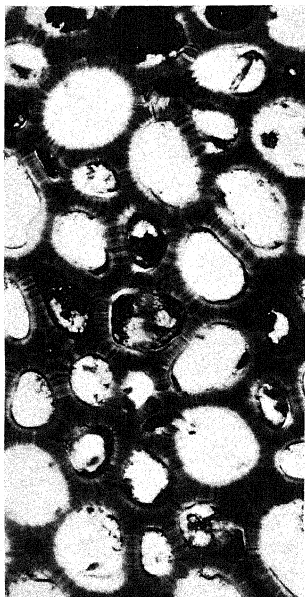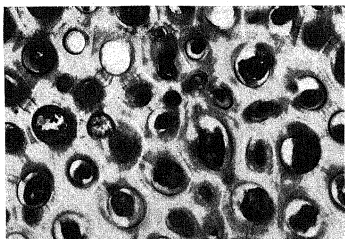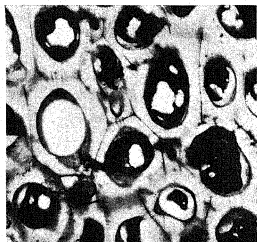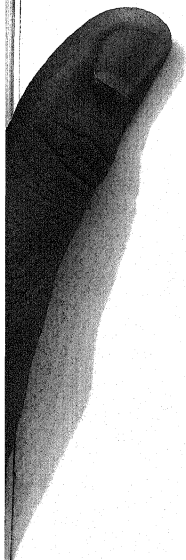
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## EXPLANATION OF PLATES XXIII, XXIV

### PLATE XXIII

All figures drawn with aid of Bausch and Lomb camera lucida, using Spencer
1.8 mm. N.A. 1.25 oil immersion objective and 6× eye-piece. All figures show a
magnification of about 620 diameters.

Fig. 1.—*D. discolor:* portion of transverse section of ripe seed, showing few
layers of testa, and outermost layers of cells of endosperm; protoplasmic connec-
tions thinnest and fewer in number at epidermal cells and layer adjacent to
epidermis; nucleus and inclusions very conspicuous; walls thicker in underlying
cells. This shows also apparent decrease and absence of protoplasmic connec-
tions in walls of epidermis.

Fig. 2.—*D. discolor:* elongated longitudinal cell in underlying region, at
seventh layer within epidermis (note nature and number of protoplasmic con-
nections).

Fig. 3.—*D. discolor:* portion of radial longitudinal section of ripe seed,
showing nature of lumen, inclusions, thickness of walls, and protoplasmic con-
nections.

Fig. 4.—*D. Ahernii:* portion of radial longitudinal section of ripe seed,
showing numerous protoplasmic connections traversing whole wall; similar to
*D. discolor.*

Fig. 5.—*D. ebenaster:* portion of radial longitudinal section of ripe seed,
showing reduced number and nature of protoplasmic connections, and character-
istic inclusions of cells.

Fig. 6.—*D. kaki:* portion of radial longitudinal section of ripe seed, show-
ing nature of protoplasmic connections and inclusions of cells, which have under-
gone degeneration.

### PLATE XXIV

All figures photomicrographed by the Bureau of Science, Manila, using C
Zeiss DD 4 mm. objective, N.A. 10 and compensating ocular no. 4. All figures
show a magnification of about 320 diameters.

All the photomicrographs are from radial longitudinal sections of ripe
endosperm cells showing protoplasmic connections.

Fig. 7.—*D. discolor.*
Fig. 8.—*D. Ahernii.*
Fig. 9.—*D. ebenaster.*
Fig. 10.—*D. kaki.*

# ·CURRENT LITERATURE

## NOTES FOR STUDENTS

**Origin of coal.**—A number of important publications have appeared during the last twelve months dealing with the origin of coal. The earlier writers on this subject were BERTRAND, POTONIÉ, WHITE, JEFFREY, and THIESSEN. The new publications consist partly of continuations of the work of some of these investigators, and also some new contributions. The most conspicuous new contributions have been made by JEFFREY.[1] The memoir summarizes JEFFREY'S life work on the origin of coal in obtaining the following conclusions. He believes that coals are the results of allochthonous sedimentation in open water. It follows that he rejects any theory according to which coals are derived from autochthonous or *in situ* deposition. JEFFREY also rejects the sapropelic hypothesis of the origin of coal, and thinks that the so-called sapropelic coals are not the consequence of the extreme disintegration of vegetable materials under water, but are derived from fine materials deposited under tranquil conditions of sedimentation in open water. According to JEFFREY, algae are not an important constituent either of existing open water deposits or of so-called sapropelic coals, such as oil shales and cannels, and the bodies interpreted as algae in cannels and oil shales are believed to be entirely spores of vascular cryptogams, etc. He considers the bituminous coals to be the result of aquatic transport and deposition, and thinks that anthracites present only a further stage of devolatilization in bituminous coals. He thinks that coking coals are predominantly of woody origin, and that in large measure their quality depends upon the proportion of wood present.

JEFFREY's book, which appeared a few months after the publication of the memoir, devotes several chapters to the origin of coal. His discussions are intended for a wider audience, and are therefore of a more popular character. A great many illustrations make the reading very easy, and the style of the book adds to the attractiveness of the study. The following coals are examined, with regard to their origin: cannel, bituminous, transitional, coking, anthracite,

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[1] JEFFREY, E. C., The origin and organization of coal. Mem. Amer. Acad. Arts & Sciences **15**: 1–52. *pls. 13.* 1924.

———, Coal and civilization. pp. ix+178. *pl. 1. figs. 44.* New York: Macmillan Co. 1925.

and brown. Cannel coals were laid down, like oil rock, in tranquil water, and consisted to a predominating degree of spores, but associated with these is a large amount of grosser vegetable material. Bituminous coal is largely derived from the vegetative parts of plants, such as leaves, stems, roots, etc., as shown by the contents of coal balls. JEFFREY characterizes as transitional coals those which are in transit from one category to another, and which show portions similar to cannel, bituminous, and anthracite in consecutive lines. An interesting statement is made about the coking coals, which show large quantities of burned woods throughout their substance. JEFFREY thinks that the only reasonable explanation of these isolated fragments throughout the coking coals is that the deposits have been transported and not formed *in situ*. He assumes that anthracite, like the mass of other coals, on the evidence of structure has not been derived from materials originally accumulated under open water, nor in peat bogs. He explains the extremely high percentage of carbon by the heating and pressure which had been brought to bear upon the deposit through the slow convolutions of mountain making. Brown coals he classifies as those which grade almost imperceptibly into peat on the one hand and bituminous on the other. They are constituted mostly of pollen grains and wood. He restricts lignite to those brown coals which are composed wholly, or at least mainly, of modified wood.

Another authority who has written previously on the origin of coal has contributed two important papers during the last year.[2] In the first paper THIESSEN explains the origin of coal as primarily due to deposition *in situ* and especially in peat bogs, which is exactly the opposite of JEFFREY's claim. This is especially true of the bituminous coal, while the cannel coals are almost entirely of spore matter. He does not believe that pressure was of great effect in the carbonization of vegetable matter, and especially in the formation of anthracite by high temperatures, although he admits that pressure may have caused a greater compactness of anthracite and other coals of high specific gravity.

In his paper on the origin of bog head coals, he tries to show that these coals are not derived from spores, but from alga-like forms not heretofore known, and he mentions a living organism found in the salt lakes of South Australia, called *Elaeophyton*. These organisms produce a great amount of oil. They apparently do not decay easily, but are blown to the shore, where they form a rubber-like mass which is called coorongite. This mass burns with a bright hot flame and melts before burning. Distilled it yields about 70 per cent of oils. THIESSEN calls coorongite the peat stage of bog head coals.

The conclusions of JEFFREY and THIESSEN are radically opposite. JEFFREY rejects the *in situ* theory, and THIESSEN believes that coal was formed in peat

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[2] THIESSEN, R., The origin and constitution of coal. pp. 44. *pls. II.* Wyoming Hist. and Geol. Soc. Wilkes-Barre, Pa. 1924.

――――, Origin of the bog head coals. U.S. Geol. Survey, Professional paper 132–121–138. Washington. 1925.

bogs. JEFFREY does not believe in the algal origin of certain coals, and THIESSEN thinks that a great deal of our coal originated that way. Both men represent very high standards of scientific investigation and technical ability. It will be interesting to observe whose opinions will prevail in the course of time.—A. C. NOÉ.

Temperature during flower-formation.—BLAAUW[3] has studied the effects of temperature during the period of flower formation on the development of leaves, roots, and flower clusters, and on the weight of the bulbs of the hyacinth. Eleven temperatures were used, ranging from 1.5° to 35° C. The temperatures were secured by means of thermostats placed in rooms which approximated the temperature desired. Humidity was also controlled by placing in the thermostats porous evaporation tubes. In regard to the development of the leaves, flowers, and flower clusters, it was found that at first the optimum was 23°–28°, but that during the treatment this changed, so that later on the greatest development was at the lower temperature, 17°–20°. If the development after planting was included in the observations, however, the higher temperatures were as favorable as the lower or even more so. This shifting of the optimum temperature from a higher to a lower degree is explained on the basis of cell division and cell extension requiring different optimum temperatures, a higher temperature being more favorable for cell division, and a lower for cell extension. Although after planting the bulbs kept continually at a higher temperature equaled or exceeded in development those kept continually at a lower temperature, a treatment which involved keeping the bulbs for a time at a higher temperature and then transferring them to a lower gave the most favorable results. In the case of the higher temperatures, a second high optimum was observed at 35°. Some statements as to the practical application of the researches are made. As an optimal treatment, where both flowers and leaves are desired, a treatment for eight weeks at 25°–27° and then four weeks at 17° is recommended; but when the flowers are not important, a treatment of 35° for five weeks and then 17° until planting time is recommended.

One is impressed with the amount of work that must have been required to make the numerous measurements and observations, and in keeping the constant temperatures for long periods of time. The illustrations are excellent. It would be interesting to determine what internal chemical changes are correlated with the growth responses noted.—S. V. EATON.

Survival of plants through the Ice Age.—It has generally been held by botanists and geologists that the species of plants now occupying arctic and cold temperate areas migrated southward in advance of the Pleistocene glaciers,

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[3] BLAAUW, A. H., The results of the temperature during flower-formation for the whole hyacinth. I. Koninklijke Akad. van Wetenschappen te Amsterdam. 23ᵛ: 1–66. 1924.

and later migrated northward to their present areas. FERNALD[4] now presents
some very strong arguments in favor of the view that, in some instances at least,
the persistence of plant species through the Ice Age was possible and even
probable in areas free from glaciation. In a few places in eastern America,
notably in the Gaspé Peninsula of Quebec and on the Long Range of western
Newfoundland, there is an extraordinary collection of species of western
America, known nowhere else east of the Rocky Mountains. These mingle
with ordinary arctic-alpine species of widespread distribution. Along with these
remarkable disjuncts, previously described by FERNALD, are 80 pronounced
endemic species with Cordilleran affinities. The striking endemism and dis-
junct distribution on the Gaspé Peninsula are in sharp contrast with the lack
of endemics and western disjuncts in Nova Scotia and eastern Newfoundland,
where there are many species of the coastal plain, separated from the main
range of these species by glacial subsidence. This splitting off of the Nova
Scotia and Newfoundland coastal plain plants from their congeners to the south
may have taken place 25,000 years ago, but this has not been long enough to
produce any endemism to speak of. FERNALD thinks, therefore, that the
endemisms of the Gaspé flora necessitate the assumption that this flora has
existed there for a vastly long period of time; in other words, through the
Pleistocene. It is certainly significant that these endemics and also the western
disjuncts are confined to areas now recognized by the geologists to have escaped
glaciation. Adjoining glaciated areas that are similar lithologically are without
these species, in spite of the thousands of years since the recession of the ice.
Also in favor of FERNALD's view is the fact that Greenland, which is now subject
to the rigors of continental glaciation, nevertheless has 416 species of vascular
plants; more than half of these species are still found near sea level south
of the St. Lawrence, and seventy-five of these species are typical plants of
temperate eastern America, thus showing that even the plants of temperate
climates may grow close to a continental ice sheet. The considerations so well
assembled in this paper make one wonder whether the plants now residing in
temperate America had to migrate so far south to escape glacial rigors as we
have commonly supposed.—H. C. COWLES.

**Chemical composition of sweet corn.**—In picking sweet corn for the table
or for canning, of course it should be picked when its chemical composition is
such that it is the most palatable. In experimental work also, it may be necessary
to pick ears of the same stage of ripeness. The nail test has frequently been used
to predict the chemical composition of sweet corn. In applying this test, the
thumb-nail is forced into the kernel, and if the exudate is milky, the corn is con-
sidered to be in the milk stage. This is the best edible stage. APPLEMAN[5] has

[4] FERNALD, M. L., Persistence of plants in unglaciated areas of boreal America.
Mem. Amer. Acad. Arts & Sciences **15**: 241–342. 1925.

[5] APPLEMAN, C. O., Reliability of the nail test for predicting the chemical composi-
tion of green sweet corn. Jour. Agric. Res. **21**: 817–820. 1921.

tried to determine how closely the chemical composition of sweet corn can be predicted by the nail test. The test was applied at different stages of ripening and at the same time samples were taken for analysis. Two crops were thus tested, one ripening in August, the other in the cool autumn. It was found that the reliability of the nail test was very much affected by the rate of ripening and by the rate of water loss by evaporation. In the case of the late crop, the ripening processes took place more slowly, and less water was lost during the ripening than in the case of the early crop. Consequently, there was less variance in the chemical composition of the kernels of each stage of ripening of the late crop, than in the corresponding stage of the early crop. The nail test becomes then a fairly reliable means of predicting the chemical composition of sweet corn, especially when applied in the case of sweetcorn ripening slowly in the autumn. —S. V. EATON.

**Root pressure exudates.**—An account of the constituents of vine sap is given by PRIESTLEY and WORMALL,[6] the data having been published previously by WORMALL.[7] Over 90 liters of exudate were analyzed after collection under sterile conditions. The solid matter constituted 1.56 gm. per liter, of which the inorganic salts formed 0.56 gm. and the organic solids 1.00 gm. Among the organic substances found, reducing sugars were most abundant, glucose 0.29 gm. per liter, and fructose 0.04 gm. per liter. Only a small quantity of non-reducing sugar was found (0.008 gm. per liter of sucrose). Four dibasic organic acids were isolated, oxalic, succinic, tartaric, and malic. Traces of another unidentified acid were found. A very small amount of organic nitrogen occurred in the sap, the smallness of which served as an excuse to mention improbable growth hormones and accessory vitamines. The presence of several enzymes, diastase, peroxidase, and catalase, accounts more satisfactorily for the presence of this constituent.—C. A. SHULL.

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[6] PRIESTLEY, J. H., and WORMALL, A., Solutes exuded by root pressure from vines. New Phytol. 24:24–38. 1925.

[7] WORMALL, A., The constituents of the sap of the vine (*Vitis vinifera* L.). Biochem. Jour. 18:1187–1202. 1924.

# GENERAL INDEX

Classified entries will be found under Contributors and Reviewers. New names and names of new genera, species, and varieties are printed in **bold-face** type; synonyms in *italics*.